

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

1/61

FIGURE 1 (SHEET 1)

SEQ ID NO: 6

1 TGAAGCTCAGCTGATGCGAGCCCGTTGGAGTGGAACGTCATTGCCGGGAACGAGCAGTC 60
61 GCCGTCGAGCCCTAGTGAAGTGGCGCCCTGCATCCGATGTCCTTCCTCCAGGCTTAC 120
121 ATGATTACCTGAAGTTTAATAAGTAAGACCATGAATTAAGCATTTCTTAATGAAGCGT 180
181 TCAAGAGTGAGAGAATGTCATAGAAATAATGATTTTAAAGTTATGTCTATTAACTG 240

SEQ ID NO: 1 M S I N L

241 ACTGATATATATATTACCTCCTAGTAATGCAAGAGTGTTGTGGAGCAGAGA 300
T V D I Y I Y L L S N A R S V C G K Q R
301 AGCAAGCACTGATTTCTTGTCTCACCCTAAGCATTAAGGATAAGCCACATCAGT 360
S K Q L Y F L F S P K H Y W R I S H I S
361 CTACAAGAGTTTCAATACAAACATATAAGATGTAATGACCAAAAGTGAAGCACAT 420
L Q R G F H T N I I R C K W T K S E A H
421 TCTTGAGTAAGCACTGTTACTCTCCAGCAACCATGTTTACATATTGGATTGAAA 480
S C S K H C Y S P S N H G L H I G I L K
481 CTAGCACTTCTGCTCCCAAGGACTTACAAAAGTGACATTTGTATGTCCTCCGATTA 540
L S T S A P K G L T K V N I C M S R I K
541 AGTACTTGAAGTCTGTTCAAAAGGCTGTTTGGCAATCAAAATGAATGATTTCAAGT 600
S T L N S V S K A V F G N Q N E M I S R
601 TTAGCTCAATTTAAGCCCAAGTCCCAATTTTAAGAAAGTATCGGATAGTGCTGTTA 660
L A Q F K P S S Q I L R K V S D S G W L
661 AAACAGAAAACATCAACAAAGCCATCAATCTGTGAATAATATAGTACAAATCAGCA 720
K Q K N I K Q A I K S L K K Y S D K S A
721 GAAAAGAGTCTTTCCAGAGAGAAAAGTCAATTAAGACAAAGAGAAGATATAGT 780
E K S P F P E E K S H I I D K E E D I G
781 AAACGAGTCTTTTCATTACACAAGTCTATTAACCAAAAATTTGAGACTCATTTCTAC 840
K R S L F H Y T S S I T T K F G D S F Y
841 TTTTATCAATCATATTAATTCATATTTCAAACGTAAAGGAAAAATGCTCAACAAAAG 900
F L S N H I N S Y F K R K E K M S Q Q K
901 GAAATGAACATTTCCGGACAATCAGAAGTGAAGATAAAAAAGTAGAAGAGGGAAA 960
E N E H F R D K S E L E D K K V E E G K
961 TTAAGATCTCAGATCTGGCATCTGGCTTATAAGCCAGGCTCAGAATCTGTACATACG 1020
L R S P D P G I L A Y K P G S E S V H T

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

2/61

FIGURE 1 (SHEET 2)

1021 GTGACAAGCCTACAGTCCTTCTGCGATACCTGATGTTCTTCAAGTTCAACTAACA 1080
V D K P T S P S A I P D V L Q V S T K Q
1081 AGTATTGCTAATTTCTTCTCTGTCGCCACGGAAGGTGTACAGCTTTAGTGGTTAT 1140
S I A N F L S R P T E G V Q A L V G G Y
1141 ATTGGTGACTTGTCCCAATAAAGTATGATTCAAAAGTCAAGTCAAGAAGACAGAA 1200
I G G L V P K L K Y D S K S Q S E E Q E
1201 GAGCCTGCTAAACTGATCAGGCTGTCAAGCAAGACAGAAATGCAGAGAGAAAAAGCGT 1260
E P A K T D Q A V S K D R N A E E K K R
1261 TTATCTCTCAGCGAAGAAAGATTATCGCAAGGGTGAATGATTAACAGGACCCGGCA 1320
L S L Q R E K I I A R V S I D N R T R A
1321 TTAGTTCAGGCATTAGAAGAACAACTGACCCAAAGCTCTGCATTACTAGGGTTGAAGA 1380
L V Q A L R R T T D P K L C I T R V E E
1381 CTGACCTTTCATCTTCTAGAATTTCTGAAGGAAAGAGAGTGCGCTGCAAGGAAGAATT 1440
L T F H L L E F P E G K G V A V K E R I
1441 ATTCATATTATTACGACTGAGACAAATTAAAGATGAAGTCTTCAAGGCTGCAGTTAGA 1500
I P Y L L R L R Q I K D E T L Q A A V R
1501 GAAATTTGGCCCTAATTGGCTATGTGATCCAGTGAAGGAGAGGAATCCGAATTCTC 1560
E I L A L I G Y V D P V K G R G I R I L
1561 TCAATTGATGGTGAGGACACAGGGCGGTGCTCTCCAGACCCTACGAAAATTAGTT 1620
S I D G G G T R G V V A L Q T L R K L V
1621 GAACCTTACTCAGAAGCCAGTTCATCAGCTCTTGATTACATTTGTGTTGTAAGCACAGGT 1680
E L T Q K P V H Q L F D Y I C G V S T G
1681 GCCATATTAGCTTTCATGTTGGGTTGTTTCATATGCCCTTGATGAATGTGAGAACTT 1740
A I L A F M L G L F H M P L D E C E E L
1741 TATCGAAATTAGATCAGATGATTTTCACAAAATGTCATTGTTGGAACAGTAATAATG 1800
Y R K L G S D V F S Q N V I V G T V K M
1801 AGTTGAGCCATGATTTTATGACAGTCAACATGGGAAAACATTCTTAAGGATAGGATG 1860
S W S H A F Y D S Q T W E N I L K D R M
1861 GGATCTGCACTGATGATTGAACAAGCAAGAAACCCACATGTCTTAAGGTAGCTGCTGTA 1920
G S A L M I E T A R N P T C P K V A A V

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

3/61

1921 AGTACCATAGTAATAGAGGGATAACACCAAGCTTTGTGTTGAGAACTATGTCAT 1980
S T I V N R G I T P K A F V F R N Y G H

FIGURE 1 (SHEET 3)

1981 TTCCCTGGAATCACTCTCATTTATTTGGAGGCTGTCAGTATAAAATGTGGCAGGCCATT 2040
F P G I N S H Y L G G C Q Y K M W Q A I
2041 AGAGCCTCATCTGCTGCTCCAGGCTACTTTGCAGATATGCATTGGGAATGATCTTCAT 2100
R A S S A A P G Y F A E Y A L G N D L H
2101 CAAGATGAGGTTTGCTTCTGAATAACCCCTCGGCATTAGCTATGCATGAGTGAATGT 2160
Q D G G L L L N N P S A L A M H E C K C
2161 CTTGGCCAGATGTGCCGTTAGAGTGCATAGTATCCCTGGGCACCTGACGTTATGAGAGT 2220
L W P D V P L E C I V S L G T G R Y E S
2221 GATGTGAGAAACACGCTAACATACACAGCTTGAATACTTAACCTTCTAATGTATCAAC 2280
D V R N T V T Y T S L K T K L S N V I N
2281 AGTGCTACAGATACAGAGAAGTCCATATAATGCTGATGGCCCTGTACCTCCGACACC 2340
S A T D T E E V H I M L D G L L P P D T
2341 TATTTAGATTCAATCCTGTATATGTGTAACAACATACCTCTAGATGAAGTCGAATGAA 2400
Y F R F N P V M C E N I P L D E S R N E
2401 AAGCTGATCAGCTGCAGTTGGAAGGTTGAATAACATAGAAAGAATGAACAAAAATG 2460
K L D Q L Q L E G L K Y I E R N E Q K M
2461 AAAAAAGTTGCAAAAATATTAGTCAGAAAAAACAACTCTGCAGAAAAATTAATGATTGG 2520
K K V A K I L S Q E K T T L Q K I N D W
2521 ATAAATTA AAAA CTGATATGTATGAAGACTTCATTCTTTCAAAATTGTGATGAGTA 2580
I K L K T D M Y E G L P F F S K L -
2581 TATGCTTATGTTCTCATTAATGAAGTCTGTTTAGAAGATCAACCATTCATTAAGGAA 2640
2641 TTGTGGGTTTCGACATGAGTTAACTTTGAATAACGTATGAATTCGAGAAATCCTGA AAA 2700
2701 AGACGCTCTCAACCAAGCTTGCAATAGCACAGAGAATATCTTGTTACAGAAATTCATAT 2760
2761 GGGAACTAGGCTTTTAAGATGTTAATAATTAGCTAAGCTTTAGTAACCTTAAGTGTGCTA 2820
2821 GTAGATTTAGTAGATATGTTGTTATATGTTGATGTTGAAATATATTAATATATG 2880
2881 TGCCGAACAAGAAACCGAAAGCTATATTTGTAAGTGTATTTTAACTTTAGTCCCTCATAAT 2940
2941 CATGTTGAATTTATGTGATCATGATTTTATTTCAATATGGAAGAAAGCTAATTTCTTTAA 3000
3001 ATTTACATTACCTAATATTTCTCACTAGCTATGTTCTCCAATCCACACTGCCTTTTATTGT 3060

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

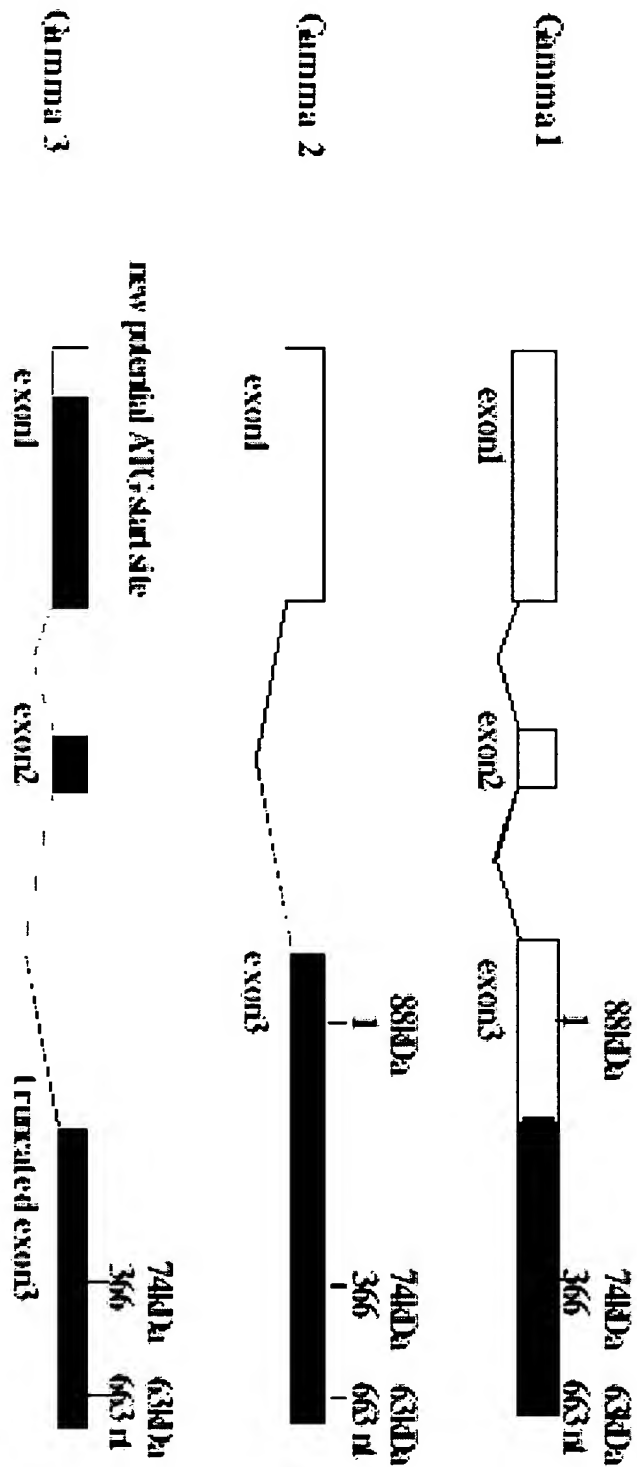
Gordon F. Sieckmann, Phone 314-621-5070

4/61

3061 AATATCATCTAATAGATGCAGAAAATGGAATTTCTCTATTAAAGTATTTACATTTG 3120
3121 ACATAAAAAGAACCGATACAGTTTCTATTGAGATATGTTATTTTACATTGTTGG 3180
3180 TTAATAAAGTGAAGTTCAGTCACCACTTTTACCCCTGAAATTTCAAGATAATGCTA 3240
3241 TATTACTTTTCCAGATCTAACACTAGCTTATCTTCCCTGTATATAAATGTTGAAGT 3300
3301 TACTGAGAGATATTCCTATCATTTACAAAAATAAATAATTAAATAATCTGTTGTTAA 3360
3361 AGGCTAATGTCATTTTAAATAATTATTTTGTTCATAATGAGCTCCCTTTAGCCTTTGA 3420
A

FIGURE 2

iPLA₂γ Splice Variants



Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.
Docket No.: 15060-42
Gordon F. Sieckmann, Phone 314-621-5070

6/61

Fig 3 Splice Variants of lPLA₂ γ

```
SEQ ID NO: 3 1 TGAAGCTCAGCTGATGCAGGCGGTTGGAGTGAGCTCATTCGCCGGAACGAGCGAGTCGCCGCTGCAGCCCTAGTGAC 80
SEQ ID NO: 4 2 TGAAGCTCAGCTGATGCAGGCGGTTGGAGTGAGCTCATTCGCCGGAACGAGCGAGTCGCCGCTGCAGCCCTAGTGAC
SEQ ID NO: 5 3 TGAAGCTCAGCTGATGCAGGCGGTTGGAGTGAGCTCATTCGCCGGAACGAGCGAGTCGCCGCTGCAGCCCTAGTGAC
SEQ ID NO: 2 M Q A G W S G R H C R E R A S R R C S P S D

| Exon 2

1
81 | Exon 3 | Exon 5 160
1 TCGCGCCTGCATCCCGGATTGTCTTCTCCTCCAAGGCTCTACATGATTACCTGAAGTTAATAAGTAGACCATGATTATG
2 TCGCGCCTGCATCCCGG...GTAGACCATGATTATG
3 TCGCGCCTGCATCCCGGATTGTCTTCTCCTCCAAGGCTCTACATGATTACCTGAAGTTAATA...
C G L H P D C L L Q G L H D Y L K F N N

residues 1-32 of SEQ ID NO: 1 161
1 GCATTCTTAAATGAAGCGTTCAAGAAGTGAGAGAATGTCATGAAAATAATGATTTTAAAGTTATGTCTATTAATCTG M S I N L
2 GCATTNTTAAATGAAGCGTTCAAGAAGTGAGAGAATGTCATNAAAATAATGATTTTAAAGTTATGTCTATTAATCTG
3

141
T V D I Y I Y L L S N A R S V C G K Q R S K Q L Y F L 220
1 ACTGTAGATATATATATTTACCTCCTTAGTAATGCAAGAGTGTGTTGTTGGAGCAGAGAGCAAGCAACTGTATTCTT
2 ACTGTAGATATATATATTTACCTCCTTAGTAATGCAAGAGTGTGTTGTTGGAGCAGAGAGCAAGCAACTGTATTCTT
3 .....TAATGCAAGAGTGTGTTGTTGGAGCAGAGAGCAAGCAACTGTATTCTT
N A R S V C G K Q R S K Q L Y F L
```

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

7/61

FIGURE 4 (SHEET 1)
Full-length iPLA₂ γ

Primers for PCR amplification of full-length 88kDa iPLA₂ γ

Sense primer M444 5'-TTTGTGACATGTCCTATTATCTGACTGTAGATA-3'

Reverse primer M458 5'-GCATAGCATGCTCACAATTTGAAGAAGATGGAAGTCC-3'

SEQ ID NO: 11
SEQ ID NO: 12

Sequence of 88kDa iPLA₂ γ :

SEQ ID NO:13 atgtctattaatctgactgtagatatatatattaccctccttagtaatgcaagaagtgt
SEQ ID NO:1 M S I N L T V D I Y I Y L L S N A R S V
tgtgggaagcagagaagcaagcaactgtatttcttctcaccctaagcattactggagg
C G K Q R S K Q L Y F L F S P K H Y W R
ataagccacatcagtcctacaagaagggttttcatacaacaataaagatgtaaatggacc
I S H I S L Q R G F H T N I I R C K W T
aaaagtgaagcacattcttcagtaagcactgttactctccaagcaaccatggtttacat
K S E A H S C S K H C Y S P S N H G L H
attggatttgaacttagcacttctgtccccaagggacttacaagaagtgaacattgt
I G I L K L S T S A P K G L T K V N I C
atgtcccgtaataaagtacttgaactctgtttccaaggctgttttggcaatcaaat
M S R I K S T L N S V S K A V F G N Q N
gaaatgattcacglttagctcaatttaagccaagttcccaaatlttaagaaagtatcg
E M I S R L A Q F K P S S Q I L R K V S
gatagtgctgtttaaaacagaaaaacatcaacaagccatcaaatctctgaaaaaatat
D S G W L K Q K N I K Q A I K S L K K Y
agtgacaatcagcagaaaaagagtcctttccagaagagaagaaagtcacattatagacaaa
S D K S A E K S P F P E E K S H I I D K
gaagaagatatagtaaacgcagtccttttcatcacacaagttctataaccacacaaaattt
E E D I G K R S L F H Y T S S I T T K F
ggagactcattctacttttatcaaatcatatattcaataattcaaacgtaaggaaaaa
G D S F Y F L S N H I N S Y F K R K E K
atgtctcaacaaaaggaatgaacatttccgggacaaatcagaacttgaagataaaag
M S Q Q K E N E H F R D K S E L E D K K

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

8/61

FIGURE 4 (SHEET 2)

gtagaaggagggaattaaagatctccagatcctggcatcctggcttataagccaggctca
V E E G K L R S P D P G I L A Y K P G S
gaatctgtacatcgytgacaagccctacaagctcctctcgtacatcctgatgttcttcaa
E S V H T V D K P T S P S A I P D V L Q
gttcaactaaacaagttatgtctaacttcttctcgtcccaaggaagtggtacaagct
V S T K Q S I A N F L S R P T E G V Q A
ttagtgtgttatattgtgtgacttgcccccaattaaagtatgttcaagaagtcag
L V G G Y I G G L V P K L K Y D S K S Q
tcagaagaacagaagagcctgtctaaactgtatcaggctgtcagcaagaacagaatgca
S E E Q E E P A K T D Q A V S K D R N A
gaggagaataaagcgttattctctcagcaggaataagattatcgcagggtgagtattgat
E' E K K R L S L Q R E K I I A R V S I D
aacaggaccgggcatagttcaggcatgaagaagaacactgaccccaagctctgcatt
N R T R A L V Q A L R R T T D P K L C I
actagggtgaagaactgacttctcatcttctaagaattcctgaaggaaaggagtggt
T R V E E L T F H L L E F P E G K G V A
gtcaaggaaagaattatccatatttattacgactgagacaataaagtgatgaactctt
V K E R I I P Y L L R L R Q I K D E T L
caggctgcagttaggaatttggccctaattggtatgtgattccagtgaaaggaga
Q A A V R E I L A L I G Y V D P V K G R
ggaatccgaattctcctaattgatgtggaaggaagggcggtgtgtctctccagacc
G I R I L S I D G G G T R G V V A L Q T
ctacgaaatagttgaacttactcagaagccagttcatcagctcttgcattatcttgt
L R K L V E L T Q K P V H Q L F D Y I C
ggtgtaagcacaggtgccatattagcttccatgttgggggtgttccatatagcccttgat
G V S T G A I L A F M L G L F H M P L D
gaatgtgaggaacttatacgaaattaggatcagatgtatttccacaaaatgtcattgtt
E C E E L Y R K L G S D V F S Q N V I V
ggaacagtaaaaatgagttggaagcatgcattttatgacagtcacaaacatggaacacatt

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

9/61

G T V K M S W S H A F Y D S Q T W E N I
ctaaggataggtggatctgcactgatgttgaaacagcaagaaacccacatgtcct
L K D R M G S A L M I E T A R N P T C P
FIGURE 4 (SHEET 3)

aagtagctgtgtaagtaaccatagtaaatagaggataaccccaagctttgtgtc
K V A A V S T I V N R G I T P K A F V F
agaactatgtcatttccctggaatcaactcattatttggaggctgtcagtataa
R N Y G H F P G I N S H Y L G G C Q Y K
atgtggcaggccattagagccctcatctgtctccaggctacttgcagaatatgcatg
M W Q A I R A S S A A P G Y F A E Y A L
ggaatgatcttcataagaatggaggttcttctgaataaccctcggcattagctatg
G N D L H Q D G G L L L N N P S A L A M
catgagttaaatgtcttggccagatgtgccgttagagtgcatagtatccctggcact
H E C K C L W P D V P L E C I V S L G T
ggacgttatggagtgatgtgagaacacggtaacatcacacaagcttgaaaactaactt
G R Y E S D V R N T V T Y T S L K T K L
tctaagtattcacacagtgctacagatcacagaagaagtcacatatatgttgatggcctg
S N V I N S A T D T E E V H I M L D G L
ttccctcctgcacacctattttagattcaatccctgttatgttgaaaacatcaccttagat
L P P D T Y F R F N P V M C E N I P L D
gaaagtcgaaatgaaaagctgcatcagctgcagttggaaggttgaaatatacatagaaga
E S R N E K L D Q L Q L E G L K Y I E R
aatgaacaaaaaatgaaaaagttgcacaaatatattgaagcaagaaaaaacactctgcag
N E Q K M K K V A K I L S Q E K T T L Q
aaaattaatgatgtgataaaatataaactgatgatgaaggaacttccattctttca
K I N D W I K L K T D M Y E G L P F F S
aaattgtga
K L -

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

10/61

FIGURE 5 (SHEET 1)

Sequence of 77kDa iPLA₂γ
starting at amino acid 101 (nucleotide 301)

Primers for PCR amplification of full-length 88kDa iPLA₂γ

Sense primer M534 5'-TGAACGTCGACATGTCCCGTATTAATAA-3'

SEQ ID NO: 14

Reverse primer M458 5'-GCATAGCATGCTCACAATTGTGAAAGAATGGAAGTCC-3'

SEQ ID NO: 12

SEQ ID NO: 16 atgtcccgctattaaagtactttgaactctgtttccaaggctgttttgccaatcaaat
SEQ ID NO: 15 M S R I K S T L N S V S K A V F G N Q N
gaaatgattccacgttttagctcaatttaagccaagttcccaaatttaagaaaagtcg
E M I S R L A Q F K P S S Q I L R K V S
gtagtggctggttaaacagaaaacatccaacaagccatcaatctctgaaaaatat
D S G W L K Q K N I K Q A I K S L K K Y
agtgaacaatcagcagaaaagagtcctttccagaagagaagaagtcacattatagacaaa
S D K S A E K S P F P E E K S H I I D K
gaagaagatatagtaaacgcagtccttttcatcacacaagttctataaccacaaaattt
E E D I G K R S L F H Y T S S I T T K F
ggagactcattctacttttcatcaaatcatattcatatttcaaacgtaaggaaaaa
G D S F Y F L S N H I N S Y F K R K E K
atgtctcaacaaaaggaaaatgaacattccgggacaacatcagaacttgaagataaaaag
M S Q Q K E N E H F R D K S E L E D K K
gtagaagagggaattaaagatctccagatccctgcatccctgcttataagccaggctca
V E E G K L R S P D P G I L A Y K P G S
gaatctgtacatacgttggaacaagcctacaagtccttctgcgatacctgatgttctcaa
E S V H T V D K P T S P S A I P D V L Q
gttccaactaaacaagtatgtctaactttcttctcgtcccaagggaagtgtaacaagct
V S T K Q S I A N F L S R P T E G V Q A

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

11/61

FIGURE 5 (SHEET 2)

ttagtagtggttatattgtgtgacttgtccccaattaaagtattcaagagtcag
L V G G Y I G G L V P K L K Y D S K S Q
tcagaagaacaggaagagcctgtctaaactgatacaggctgtcagcaagacagaatatgca
S E E Q E E P A K T D Q A V S K D R N A
gaggaagaaaaagcgttatctcttcagcgaagaaaaagattatcgcaagggtgagtattgat
E E K K R L S L Q R E K I I A R V S I D
aacaggaacccgggcattagttcagggcattaagaagaacaactgaccccaagctctgcatt
N R T R A L V Q A L R R T T D P K L C I
actagggttgaagaactgactcttcattctcttagaatttctcgaaggaaaaaggagtgct
T R V E E L T F H L L E F P E G K G V A
gtcaaggaaaaaatattccatatttattacgactgagacaataatgaagtgaactctt
V K E R I I P Y L L R L R Q I K D E T L
caggtcgcagttagaagaatttggccctaattggctatgtgacccagtgaaaggagaga
Q A A V R E I L A L I G Y V D P V K G R
ggaatccgaattctctcaattgatgtggaagaagggcggtgtgtctctccagacc
G I R I L S I D G G G T R G V V A L Q T
ctacgaaaattagttgaacttactcagaagccagttcatcagctcttggattacatttgt
L R K L V E L T Q K P V H Q L F D Y I C
ggtgtaagcacaggtgccatatattagcttcatgttggtgtttcatatgccccttgat
G V S T G A I L A F M L G L F H M P L D
gaaatggaagaactttatcgaaaattaggtatcagatgtatttccaaaatgtcatgtt
E C E E L Y R K L G S D V F S Q N V I V
ggaacagtaaaaatgagttggagccatgcattttatgacagtcacaacatgggaaaaacatt
G T V K M S W S H A F Y D S Q T W E N I
cttaaggatagtgatgtcactgtgatgtgaacacagcaagaagaacccacatgtcct
L K D R M G S A L M I E T A R N P T C P
aaggtagctgtgtgaagtaccatagtaaatagagggataacaccccaagcttltgtgttc

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

12/61

K V A A V S T I V N R G I T P K A F V F

FIGURE 5 (SHEET 3)

agaactatggtcatttctctggaatcaactctcatttgggaggtgtcagtataa
R N Y G H F P G I N S H Y L G G C Q Y K
atgtggcaggccattagagccctcatctgtgtctccaggctactttgcagaatatgcatg
M W Q A I R A S S A A P G Y F A E Y A L
ggaatgatcttcattcaagatggagggttcttctgaataacccttcggcattagctatg
G N D L H Q D G G L L L N N P S A L A M
catgagtgttaaatgtctcttgccagatgtgccgttagagtgcatagtatccctgggcaact
H E C K C L W P D V P L E C I V S L G T
ggacgttatgaggtgtgtgagaacacggttaacatcacacaagcttgaaaactaact
G R Y E S D V R N T V T Y T S L K T K L
tctaattgtatcaacagtgctacagatacagaagaagttccatataatgttgatggcctg
S N V I N S A T D T E E V H I M L D G L
ttacctcctgacacctatttagatccaatccctgtaatgttgaaaacatacctctagat
L P P D T Y F R F N P V M C E N I P L D
gaaagtcgaaatgaaagctggtcagctgcagttggaagggttgaatatacatagaaga
E S R N E K L D Q L Q L E G L K Y I E R
aatgaacaaaaaatgaaaaaagtgcacaaaatatagtcagaagaaaaaacactctgcag
N E Q K M K K V A K I L S Q E K T T L Q
aaaattaatgattgataaaataaaaactgatatgtatgaaggacttccattctttca
K I N D W I K L K T D M Y E G L P F F S
aaattgtga
K L -

FIGURE 6 (SHEET 1)

Sequence of 74kDa iPLA₂γ
starting at amino acid 122 (nucleotide 364)

Primers for PCR amplification of full-length 88kDa iPLA₂γ

Sense primer M533 5'-TCAAGTCGACATGATTTCACGTTAGC-3'

SEQ ID NO: 17

Reverse primer M458 5'-GCATAGCATGCTCACAAATTTGAAAGAATGGAAGTCC-3'

SEQ ID NO: 12

SEQ ID NO: 19 atgattcacggttagctcaatttaagccaagttcccaaatTTtaagaaaagtatcg
SEQ ID NO: 18 M I S R L A Q F K P S S Q I L R K V S
gatagtggctgttaaaacagaaaaaacatcaaaccaagccatcaaatctctgaaaaatat
D S G W L K Q K N I K Q A I K S L K K Y
agtacaaatcagcagaaaagagtcctttccagaagagaaaagtcacattatagacaaa
S D K S A E K S P F P E E K S H I I D K
gaagaagatatagtaaacgcagtccttttccattacacaagttctataaccacaaattt
E E D I G K R S L F H Y T S S I T T K F
ggagactcattctacttttatccaatcatatattcatatttcaacgtaaggaaaaa
G D S F Y F L S N H I N S Y F K R K E K
atgtctcaacaaaaggaaaatgaacatttccgggacaaatcagaacttgaagataaaaag
M S Q Q K E N E H F R D K S E L E D K K
gtagaagaggggaattaatagatctccagatccctgcacccctgcttataagccaggtca
V E E G K L R S P D P G I L A Y K P G S
gaatctgtacatacgytgacaagccctacaagtccttctgcgatacctgatgttcttcaa
E S V H T V D K P T S P S A I P D V L Q
gttccaactaaacaaagtattgtctaactttcttctcgtcccaagggaaggtgtacaagct
V S T K Q S I A N F L S R P T E G V Q A
ttagttagtggttatattgttgactgtgtcccaaattaagtatgatccaagagtcag
L V G G Y I G G L V P K L K Y D S K S Q
tcagaagaacaggaagagcctgtctaactgatcaggctgtcagcaagaagacagaatatgca

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

14/61

FIGURE 6 (SHEET 2)

aacaggaccggcggttagttcaggcattagaagaacaactgaccacaagctctgcatt
N R T R A L V Q A L R R T T D P K L C I
actagggtgaagaactgacttttcattcttctagaatttcctgaaggaaaggagtgct
T R V E E L T F H L L E F P E G K G V A
gtcaagggaagaattattccattatttaccagctgagacaattagaatgaactctt
V K E R I I P Y L L R L R Q I K D E T L
caggctgcagttgagaattttggccctaatgtgtatgtgcatccagtgaaaggaga
Q A A V R E I L A L I G Y V D P V K G R
ggaatccgaattctctcaattgtagtgtaggaacaaggcggtgtgtctctccagacc
G I R I L S I D G G T R G V V A L Q T
ctacgaaaattagttgaacttaccagaagccagttcaccagctcttgattacattgt
L R K L V E L T Q K P V H Q L F D Y I C
ggtgtaagcacagtgccatatagctttcattgttggtgtttccatatgcccctggat
G V S T G A I L A F M L G L F H M P L D
gattgtgaggaactttatcgaattaggtatcagatgtattttcacaataatgtcattgt
E C E E L Y R K L G S D V F S Q N V I V
ggaacagtaaaatgagttgagccatgcattttatgacagtcacaacatggaacaact
G T V K M S W S H A F Y D S Q T W E N I
cttaaggatagtggtgctgtcactgtagttgaacaacagcaagaacccccacatgtcct
L K D R M G S A L M I E T A R N P T C P
aaggtagctgtgtaagttaccatagtaaatagaggataacacccaagctttgtgttc
K V A A V S T I V N R G I T P K A F V F
agaaactatgttcatttccctggaatcaactctcattatttggaggctgtcagataaa
R N Y G H F P G I N S H Y L G G C Q Y K
atgtggcaggccattagagcctcatctgtctccaggctactttgcagaatatgcatg
M W Q A I R A S S A A P G Y F A E Y A L
ggaatgatcttcatcaagatgaggtttgtcttgaataaaccttggcattagctatg
G N D L H Q D G G L L L N N P S A L A M
catgagtgtaatgtcttggccagatgtgcccgttagagtgcatagtatccctgggcact
H E C K C L W P D V P L E C I V S L G T
ggacgttatgagagtgatgtgagaacacggttaacatacacaagcttgaaaactaac
G R Y E S D V R N T V T Y T S L K T K L
tctaattgtatcaacagtgctacagatagagaagaagttccatatatatgtgtgctg

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

15/61

FIGURE 6 (SHEET 3)

S N V I N S A T D T E E V H I M L D G L
ttacctcctgacacctatttagattcaatcctgtaatgtgtgaaacacataccttagat
L P P D T Y F R F N P V M C E N I P L D
gaaagtcgaaatgaaagctggtcagctgcagcttggaagggttgaaatatacagaaga
E S R N E K L D Q L Q L E G L K Y I E R
aatgaacaaaaaatgaaaaagltgcaaaaaatatttaagtcaagaaaaaacaactctgcag
N E Q K M K K V A K I L S Q E K T T L Q
aaaattaatgattgataaaatlaaaactgatatgtatgaaggacttccattctttca
K I N D W I K L K T D M Y E G L P F F S
aaattgtga
K L -

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

16/61

FIGURE 7 (SHEET 1)

Sequence of 63kDa iPLA₂γ
starting at amino acid 221 (nucleotide 661)

Primers for PCR amplification of full-length 88kDa iPLA₂γ

Sense primer M530 5'-GTAAGTCGACCAATGCTCAACAAAAAGG-3'

SEQ ID NO: 20

Reverse primer M458 5'-GCATAGCATGCTCACCAATTGTGAAAAGAATGGAAGTCC-3'

SEQ ID NO: 12

SEQ ID NO: 22 atgtctcaacaaaggaaatgaacatttccgggacaatcagaacttgaagataaaag
SEQ ID NO: 21 M S Q Q K E N E H F R D K S E L E D K K
gtagaagagggaattagatctccagatccctggcatccctgttataagccaggctca
V E E G K L R S P D P G I L A Y K P G S
gaatctgtacatagcgtggacaagcctacaagtccttctgcgatacctgatgttctcaa
E S V H T V D K P T S P S A I P D V L Q
gttccaactaaacaagtattgtctaactttcttctcgtcccaagggaaggtgtacaagct
V S T K Q S I A N F L S R P T E G V Q A
ttagtagtggtatatgtgtgactgtgtccccaattaaagtatgatccaagaagtcag
L V G G Y I G G L V P K L K Y D S K S Q
tcagaagaacaggaagagcctgtctaaactgtatcagctgtcagcaagaacagacaatgca
S E E Q E E P A K T D Q A V S K D R N A
gaggagaaaaagcgtttatctcttcagcgagaaagaattatcgcaaggtgagtattgat
E E K K R L S L Q R E K I I A R V S I D
aacaggacccgggcattagttcaggcattagaagaacaactgacccaagctctgcatt
N R T R A L V Q A L R R T T D P K L C I
actagggttgaagaactgacttttcatcttctagaatttctgaagaaaggagtggtct
T R V E E L T F H L L E F P E G K G V A
gtcaaggaaagaattatcccatatttatacgaactgagacaataatgaagatgaactctt
V K E R I I P Y L L R L R Q I K D E T L
caggctgcagttagagaatttggccctaattggctatgtggtccagtgaaagggaga

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

17/61

Q A A V R E I L A L I G Y V D P V K G R
FIGURE 7 (SHEET 2)

ggaatccgaattctctcaattgattggtggaggaacaaggcggtgtgtctctccagacc
G I R I L S I D G G T R G V V A L Q T
ctacgaaaattagttgaacttactcagaagccaggttcacagctcttgattacattgt
L R K L V E L T Q K P V H Q L F D Y I C
ggtgtaagcacaggtgccattattagcttccatgttgggtgttccatattgcccttgat
G V S T G A I L A F M L G L F H M P L D
/ gaatgtggaactttatcgaaaattaggatcagatgtatttcacaaatgtcattgtt
E C E E L Y R K L G S D V F S Q N V I V
ggaacagtaaaaatgagttggagccatgcattttatgacagtcacaacatgggaaaacatt
G T V K M S W S H A F Y D S Q T W E N I
ctaaggataggatgggattctgcactgatgtgaacacagcaagaacccccacatgtcct
L K D R M G S A L M I E T A R N P T C P
aaggtagctgtgtaagtaaccatagtaaatagaggataacacccaagcttctgtctc
K V A A V S T I V N R G I T P K A F V F
agaacctatgtcatttccctggaatcaactctcatatttgggaggtgtcagtataaa
R N Y G H F P G I N S H Y L G G C Q Y K
/ atgtggcaggccattagagcctcatctgtctgtccaggctacttgcagatatgcatgt
M W Q A I R A S S A A P G Y F A E Y A L
ggaatgatcttcacatcagatggaggttcttctgaataacccttcggcatagctatg
G N D L H Q D G G L L L N N P S A L A M
catgagtgaatgtcttggccagatgtgccgttagagtgcatagtatccctgggcact
H E C K C L W P D V P L E C I V S L G T
ggacgttatgagagtgatgtgagaacaacaggtacacatacaacagcttgaaaactaact
G R Y E S D V R N T V T Y T S L K T K L
tctaattgtatacaacagtgctacagatacagaagaagttccatataatgttgatggcctg
S N V I N S A T D T E E V H I M L D G L
ttacctcctgacacctatttagattcaatccctgtaatgtgtgaaaaacatacctctagat

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.
Docket No.: 15060-42
Gordon F. Sieckmann, Phone 314-621-5070

18/61

L P P D T Y F R F N P V M C E N I P L D

FIGURE 7 (SHEET 3)

gaaagtcgaaatgaaaagctggatcagctgcagttggaagggtgaatatagagaaga
E S R N E K L D Q L Q L E G L K Y I E R
aatgaacaaaaaatgaaaagctggcaaaaatatagtcagaagaaaaaacactctgcag
N E Q K M K K V A K I L S Q E K T T L Q
aaaatgaatggatgataaaatlaaaactgatatgtgaaggacttccattcttcca
K I N D W I K L K T D M Y E G L P F F S
aaatgtga
K L -

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

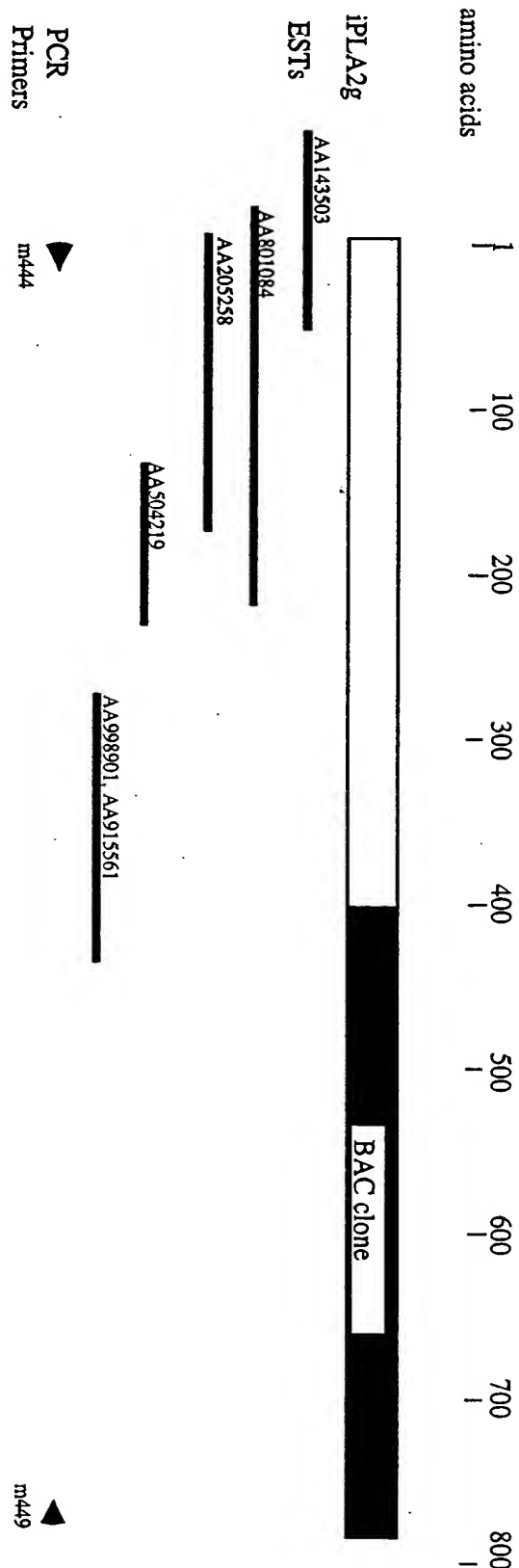
Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

19/61

FIGURE 8



Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

20/61

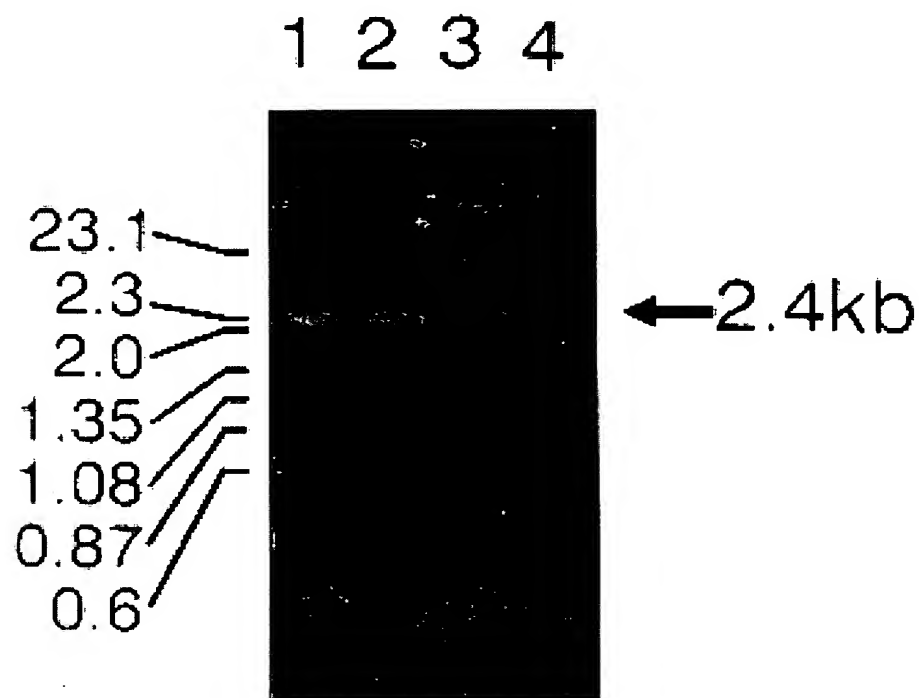


FIGURE 9

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

21/61

FIGURE 10

Potential Alternative Exon 5 Splice Variant of Human iPLA₂ γ

A. Reported Splice Sequence (gc/ag)

<u>Exon 5 (SEQ ID NOS 43-44)</u>	<u>Intron 5</u>	<u>Exon 6 (SEQ ID NOS 45-46)</u>	<u>Source</u>
...CAG CGA GAA AAG	gcaagt...ttgtag	ATT ATC GCA AGG GTG AGT	(Tanaka et al)
Q R E K		I I A R V S	BBRC 272: 320, 2000

B. Potential Splice Variant (gt/ag)

<u>Exon 5 (SEQ ID NOS 47-48)</u>	<u>Intron 5</u>	<u>Exon 6</u>	<u>Source</u>
..GAA AAG GCA AGT TGT TCA GT	gtgctt..tcgcaag	G GTG AGT	(Gross lab)
E K A S C S V		V S	JBC 275: 9937, 2000

The incidence of gc/ag splice variants like the one shown in "A" is 0.56%. The variant "A" has been reported in the literature, reported in GenBank, and cloned in our lab.

The splice variant gt/ag occurs with a frequency of 98.71% among genes. However, variant "B" iPLA₂ γ sequence has not been cloned.

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

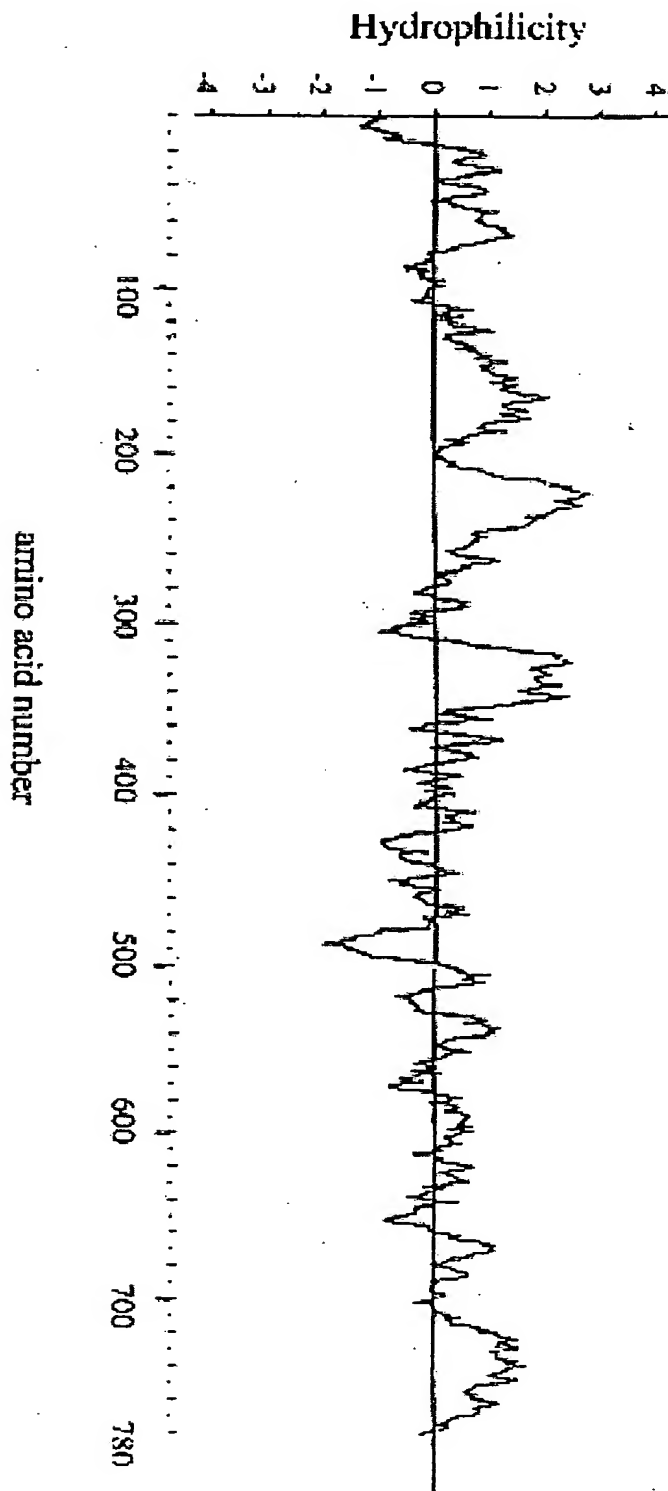
Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

22/61

FIGURE 11



Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

23/61

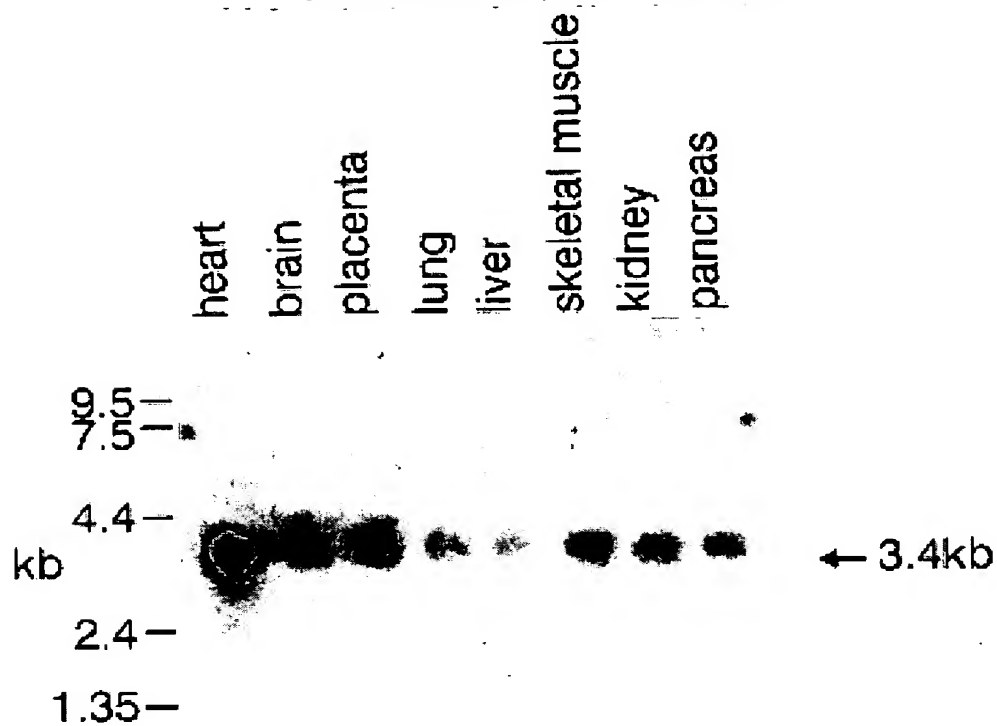


FIGURE 12

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

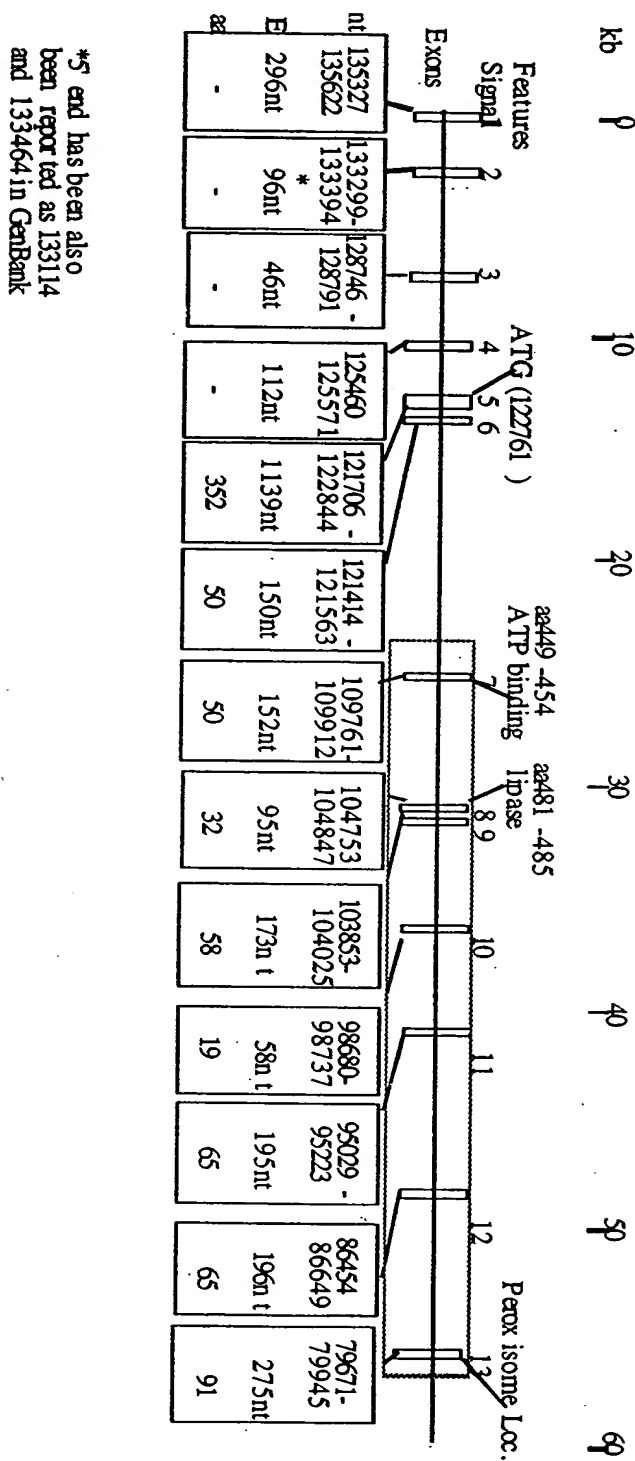
Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

24/61

FIGURE 13



Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

25/61

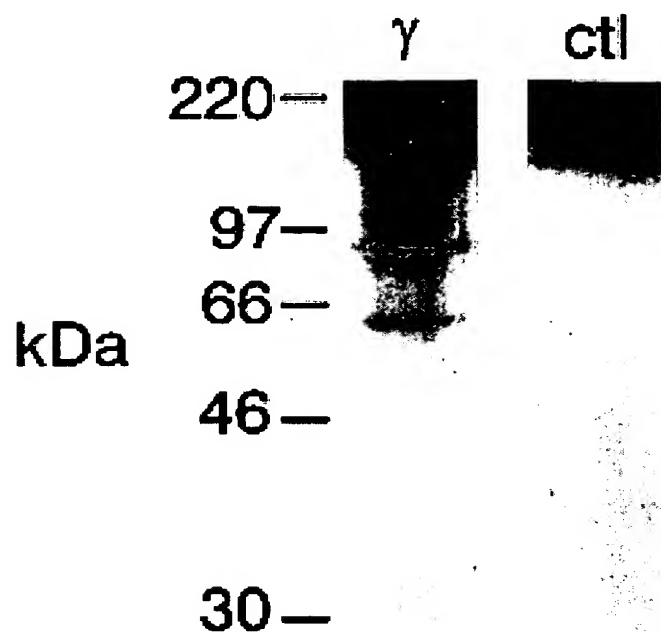


FIGURE 14

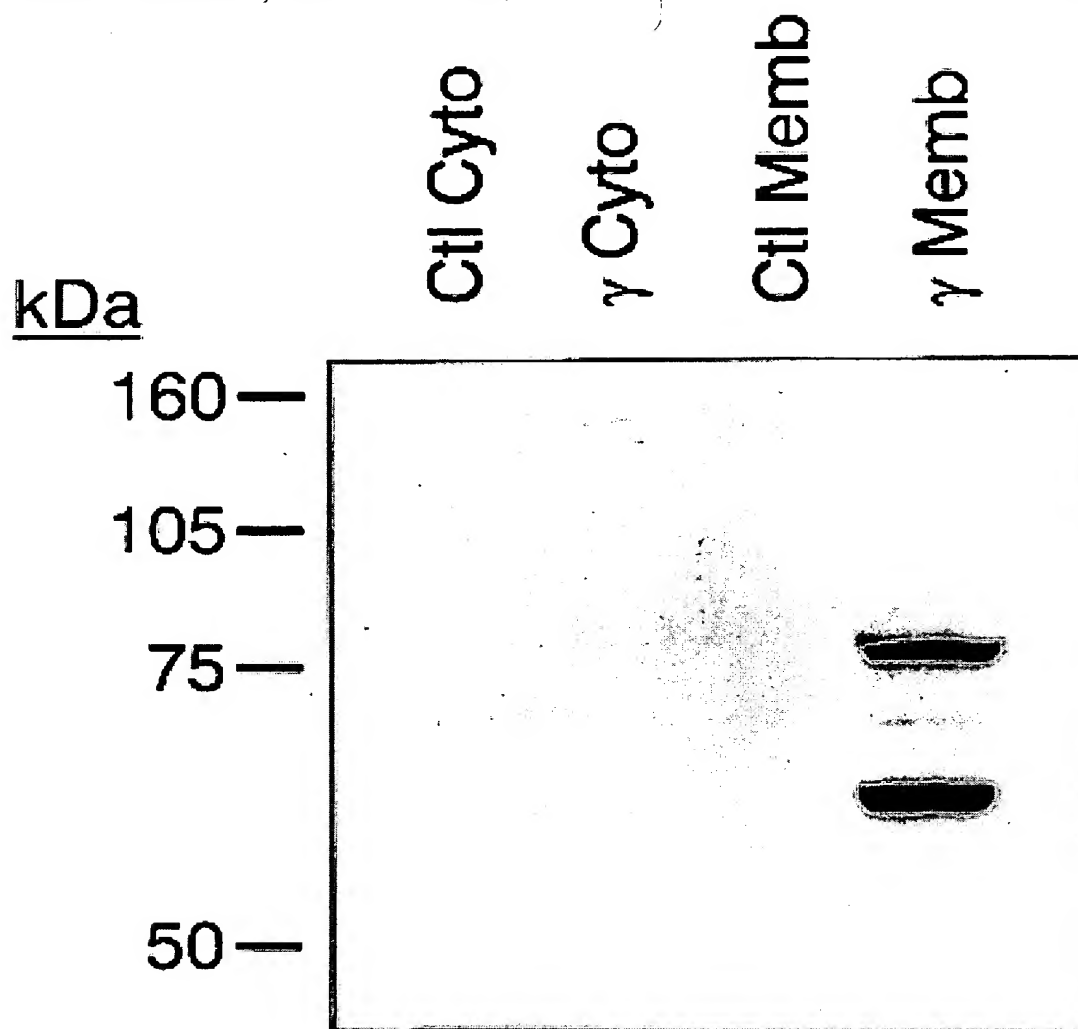


FIGURE 15

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.
Docket No.: 15060-42
Gordon F. Sieckmann, Phone 314-621-5070

27/61

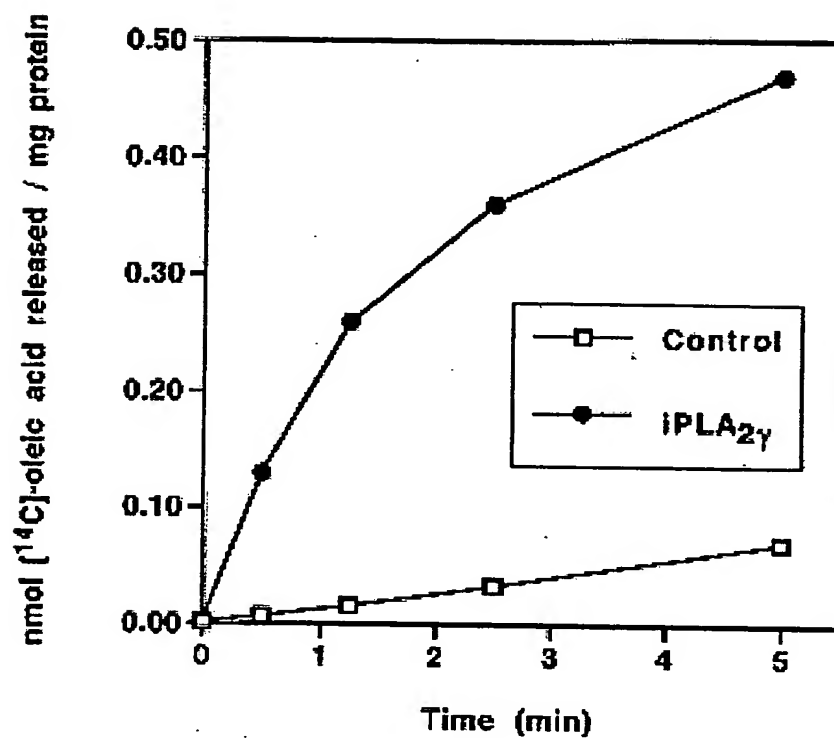


FIGURE 16

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.
Docket No.: 15060-42
Gordon F. Sieckmann, Phone 314-621-5070

28/61

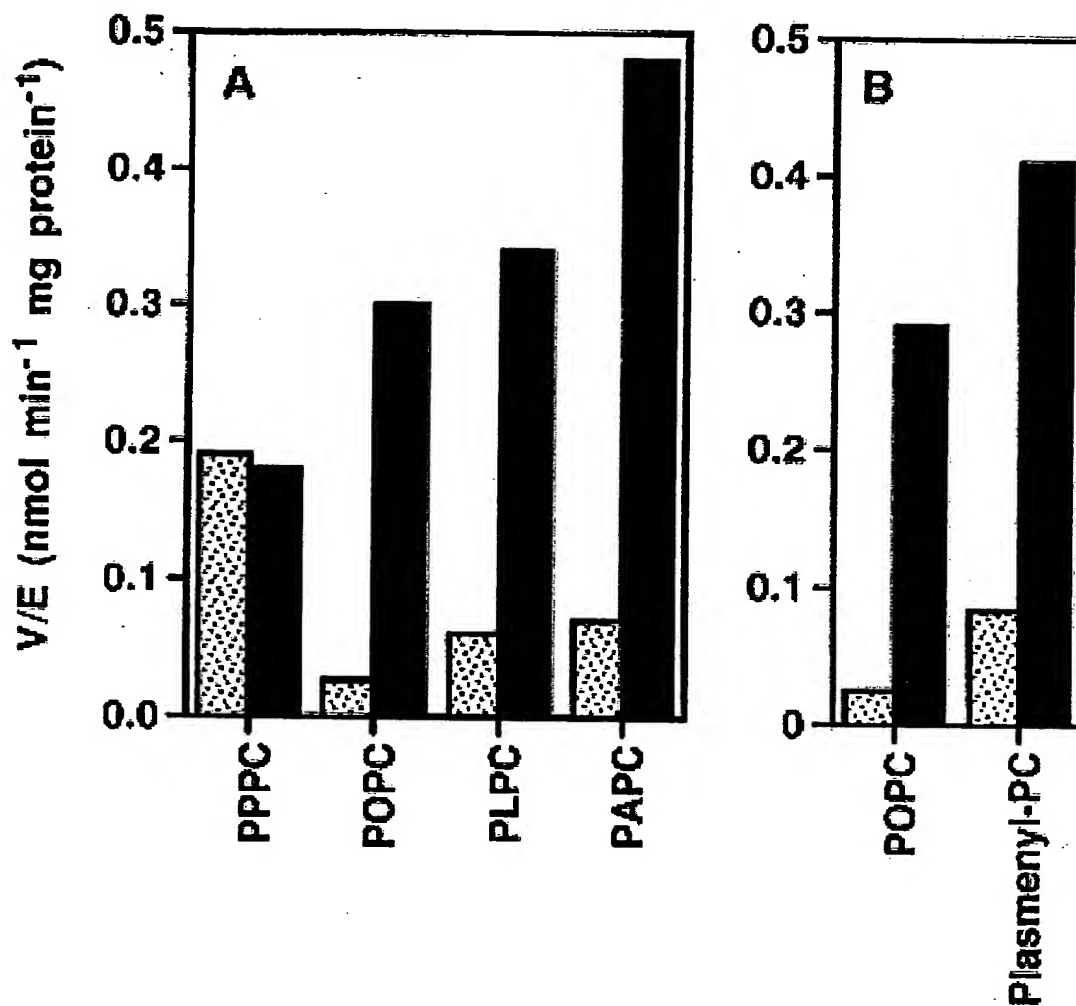


FIGURE 17

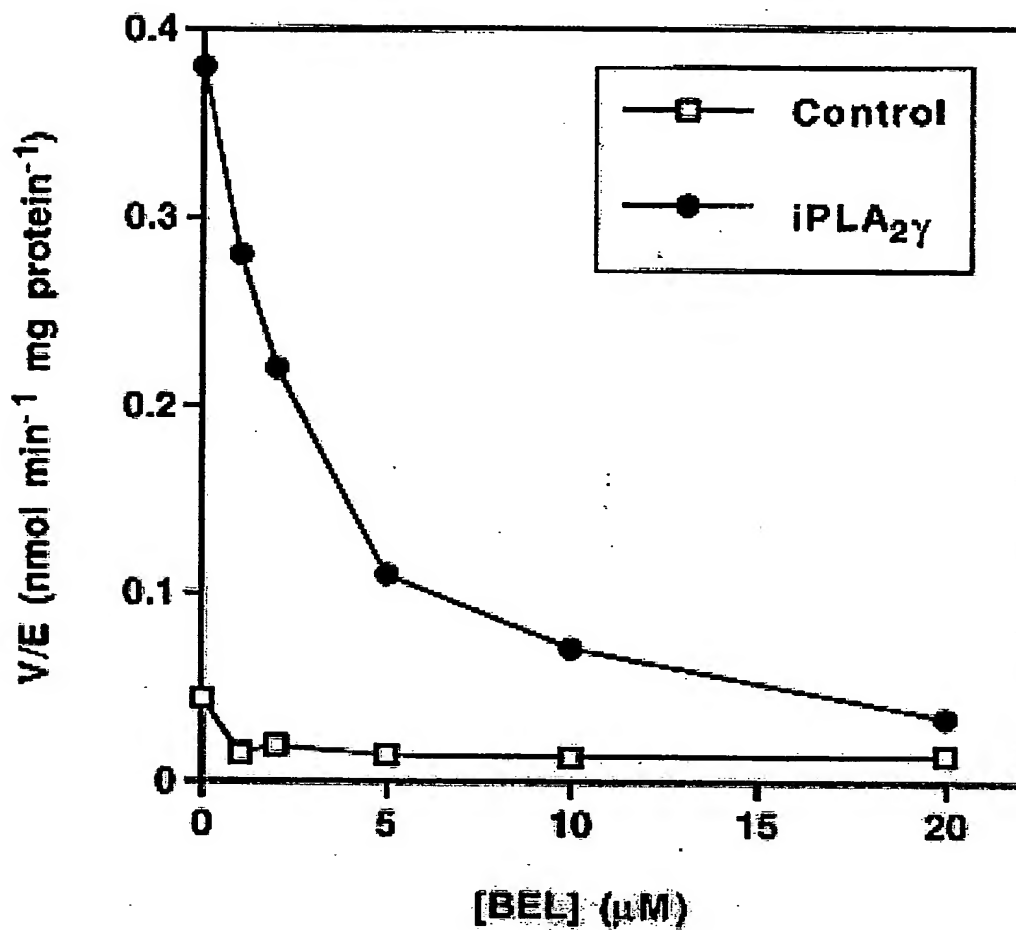


FIGURE 18

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

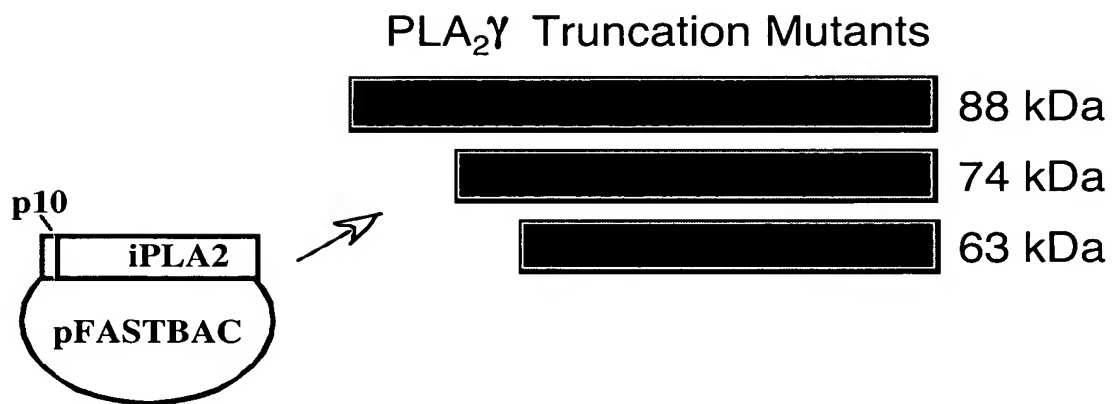
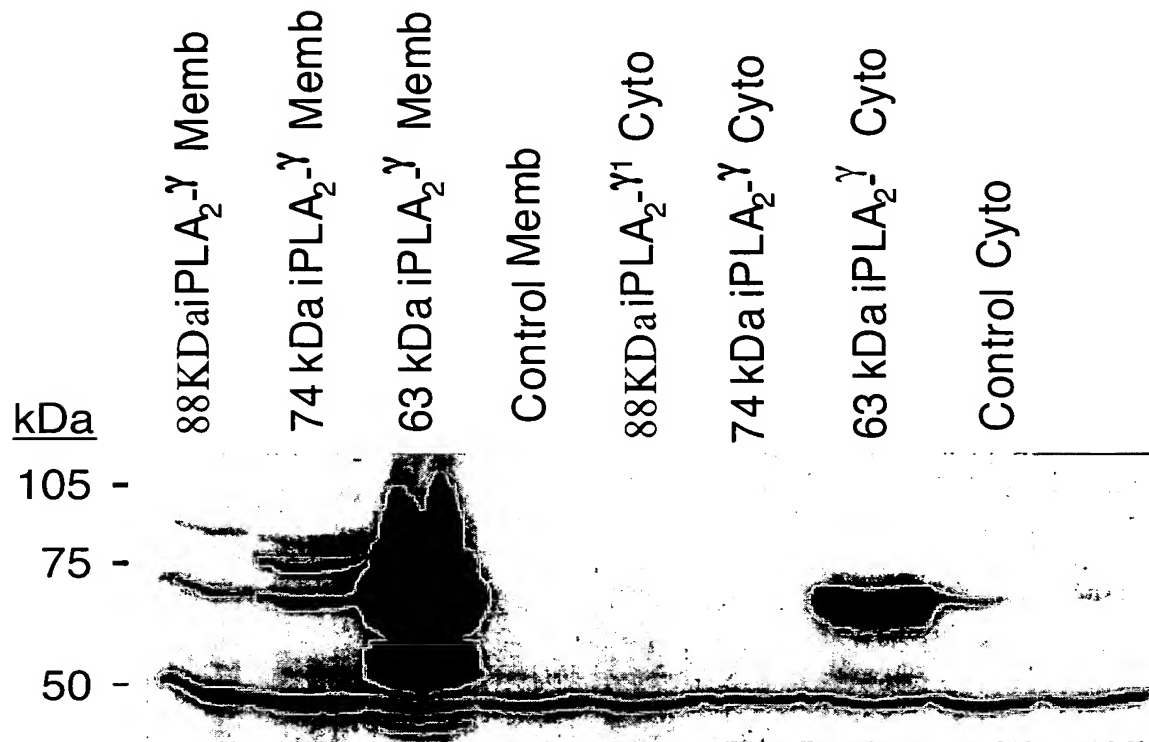
Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

FIGURE 19

30/61



Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

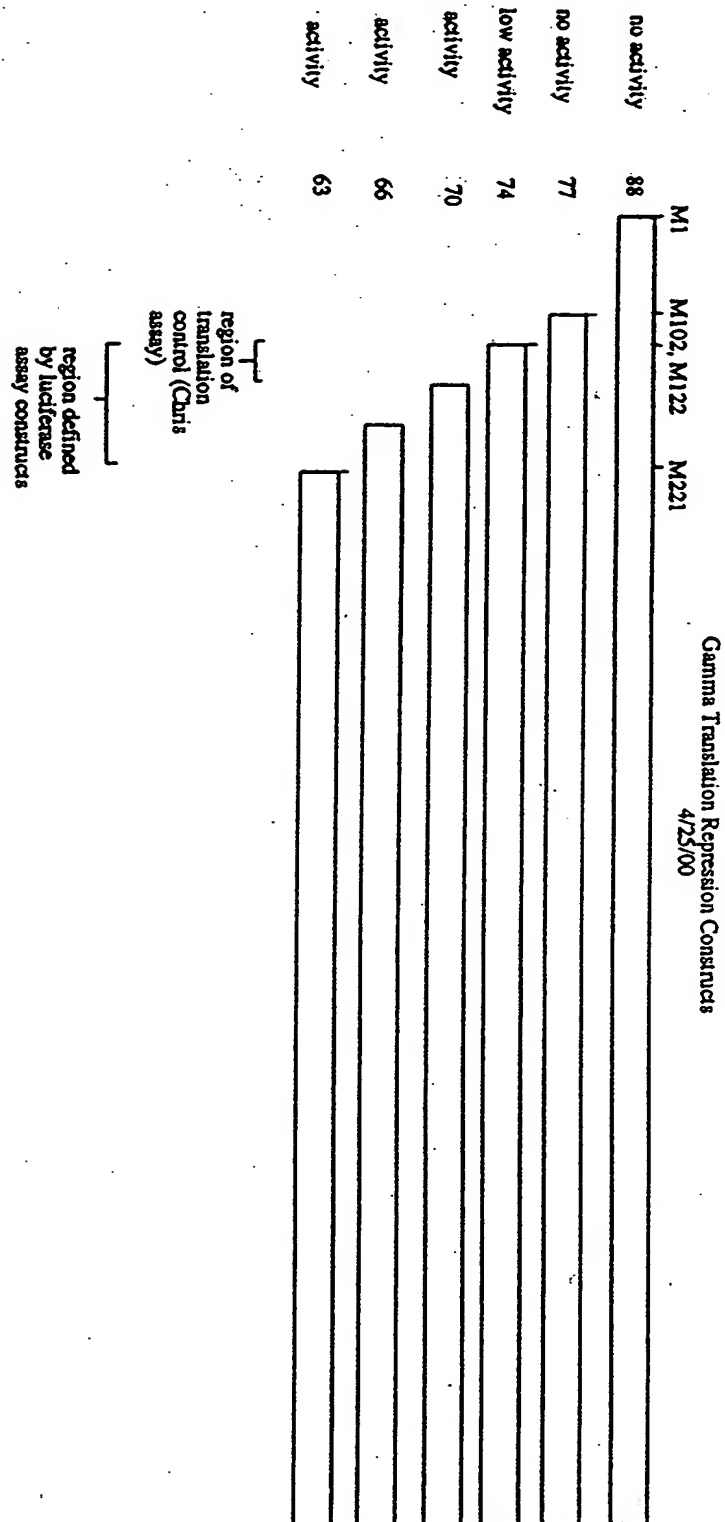
Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

31/61

FIGURE 20



Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

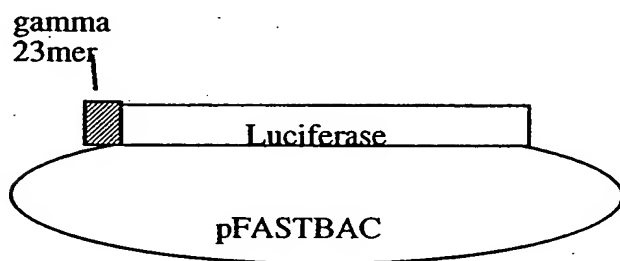
Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

32/61

FIGURE 21

Additionally, iPLA₂γ sequences were inserted by ligation of 15-23mer annealed phosphorylated oligonucleotide pairs 5' of full-length luciferase coding sequence cloned into pFASTBAC via NotI/XbaI restrictions and then luciferase activity of recombinant protein produced in the Sf9 system was subsequently measured using the Luciferase Assay System of Promega.



Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

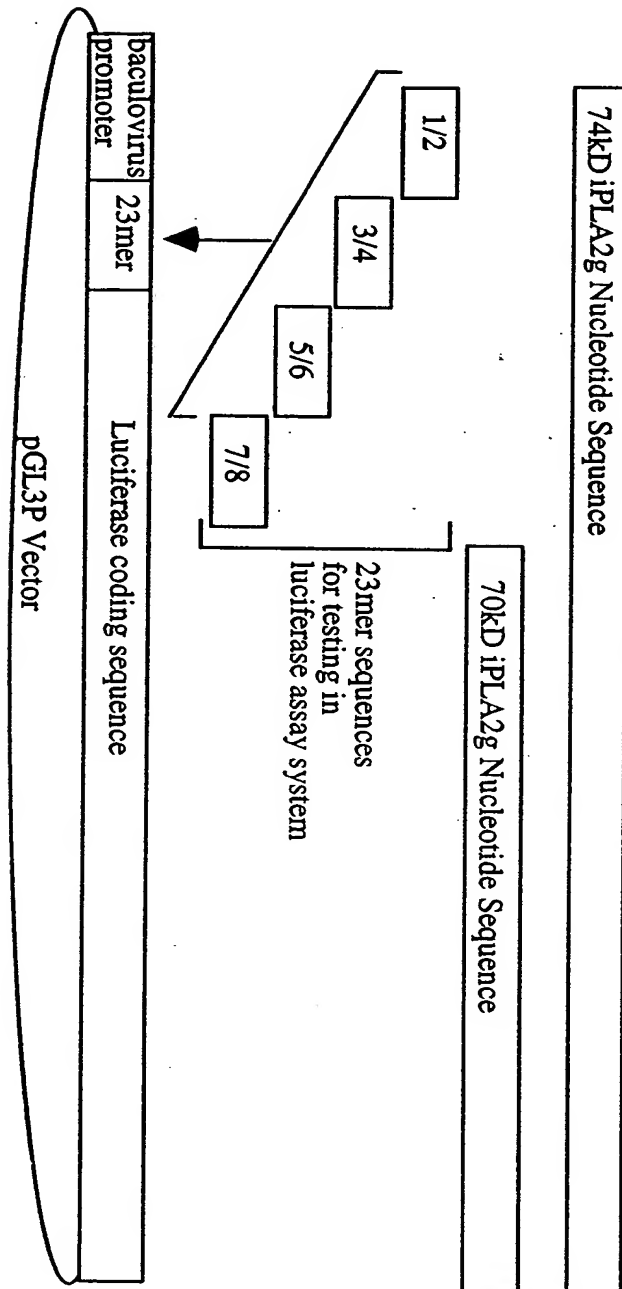
Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

33/61

FIGURE 22



Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

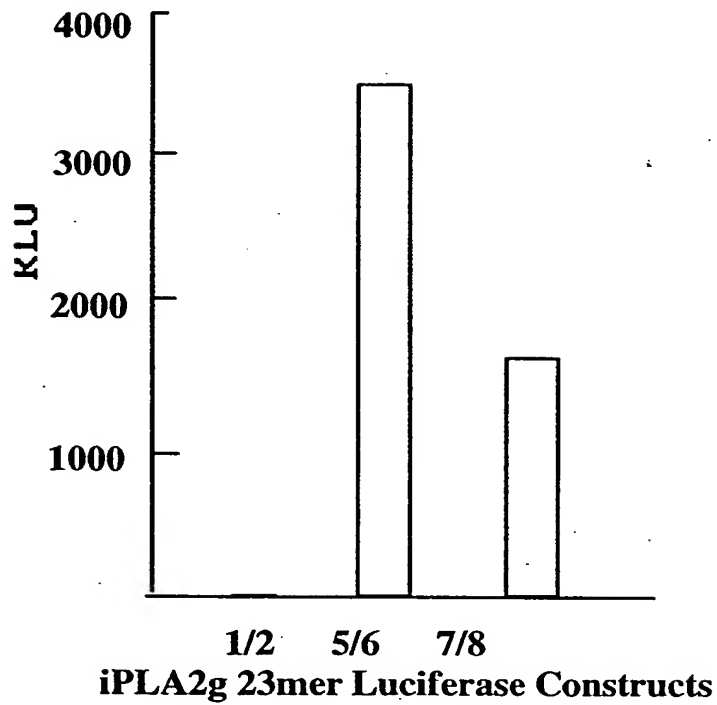
34/61

FIGURE 23. iPLA₂γ Repressor Region

Phosphorylated oligo pairs
for repression of iPLA₂γ in the luciferase expression system:

SEQ ID NO: 10	atgattccacgcttagctcaatttaagccaagttcccaattttaagaaagatcggatagtgctggttaaaacagaaaaacatcaaca
SEQ ID NO: 32	tcgacctgattccacgcttagctcaatt
SEQ ID NO: 36	ggactaaagtgcgaatcgagtttaaccgg
SEQ ID NO: 33	
SEQ ID NO: 37	tcgactaagccaagttcccaatttta
	gattcggttcaagggtttaaatccgg
SEQ ID NO: 34	
SEQ ID NO: 38	tcgacgaaagtatcgatagtgctgg
	gctttcatagcctatcacccgaccgg
SEQ ID NO: 35	
SEQ ID NO: 39	tcgacttaaacagaaaaacatcaaca
	gaatttgtctttttgtagttgtccgg

FIGURE 24



Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

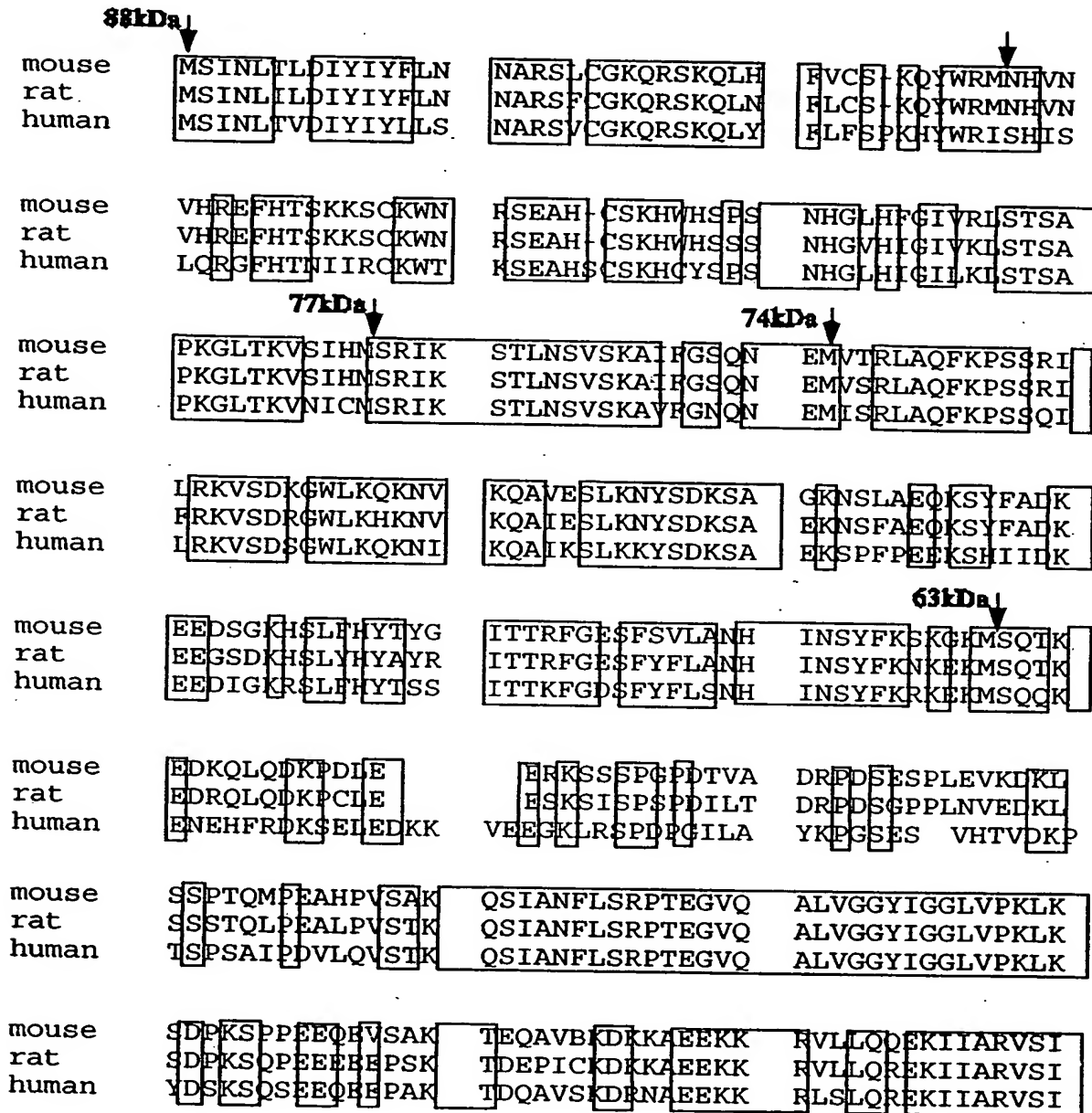
Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

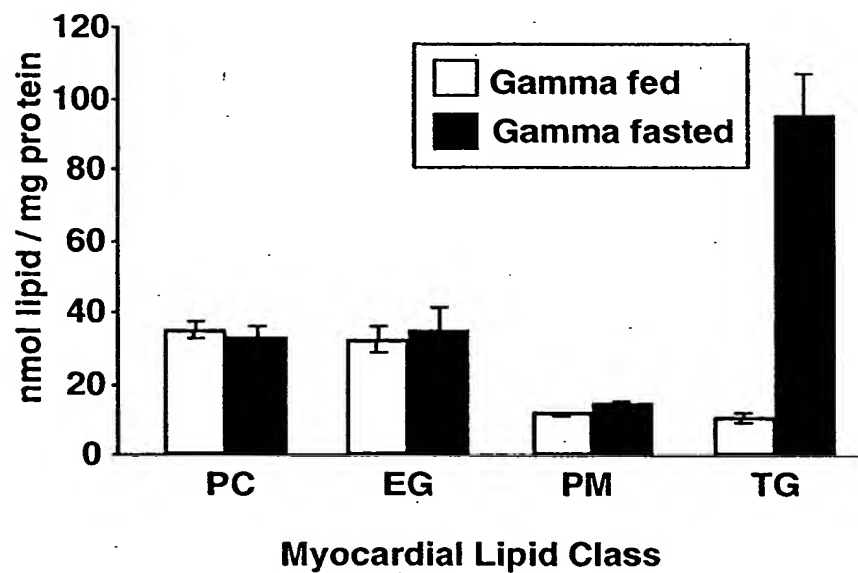
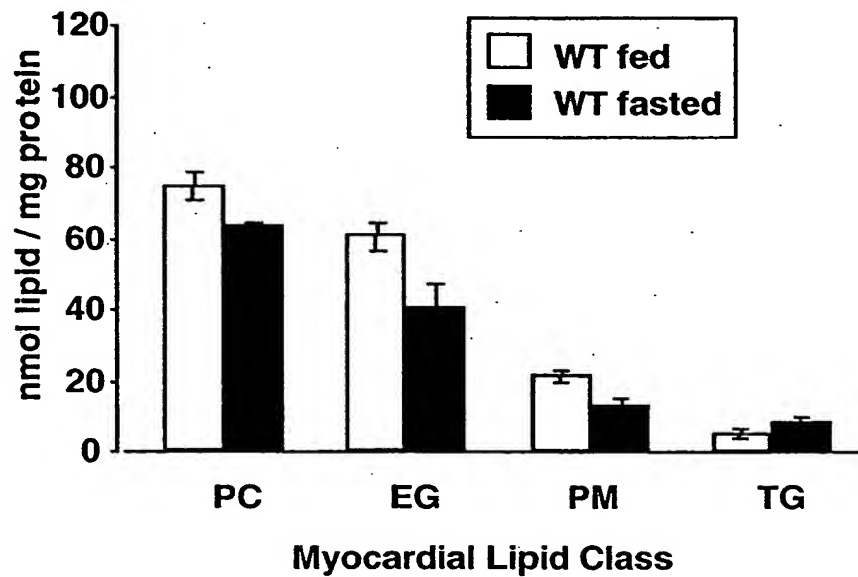
36/61

FIGURE 25



SEQ ID NO: 40 = N terminal 353 amino acids of mouse iPLA₂ γ
 SEQ ID NO: 41 = N terminal 353 amino acids of rat iPLA₂ γ
 SEQ ID NO: 42 = N terminal 359 amino acids of human iPLA₂ γ

Fig. 26 Myocardial TAG Content of Fasted WT vs iPLA₂ γ Transgenic Mice



PC = Phosphatidylcholine
EG= Ethanolamine Glycerophospholipids
PM= PLasmalogen
TG= Triacylglyceride

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

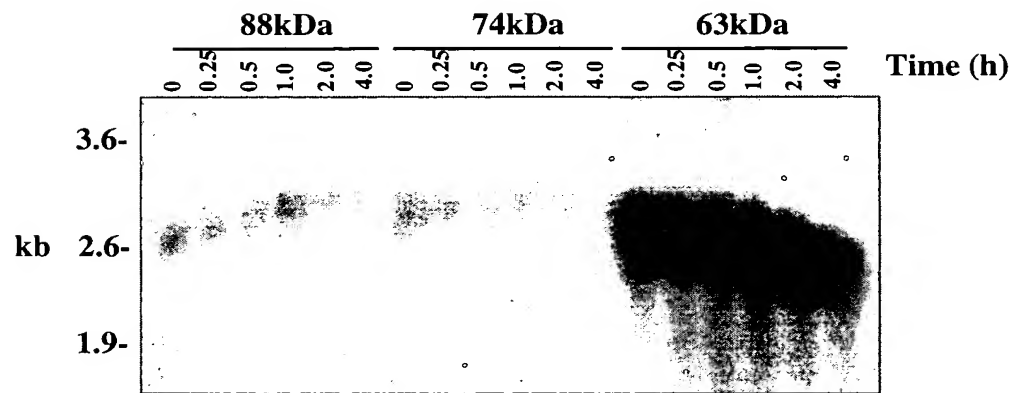
Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

38/61

FIGURE 27



Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

39/61

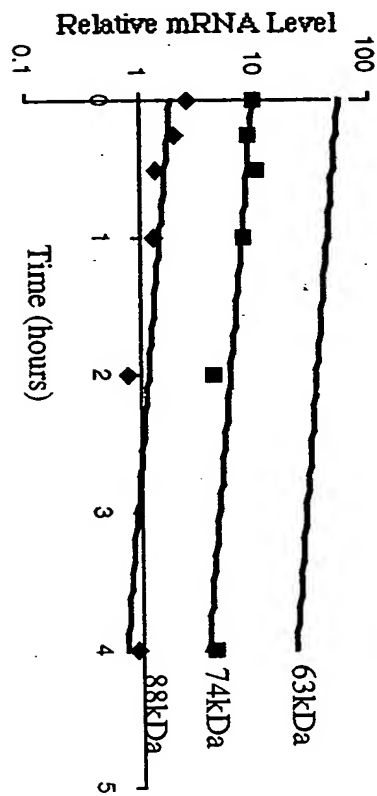


FIGURE 28. Quantitative PCR analysis of RNA stability of truncated iPLA₂ γ S19 Expression

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

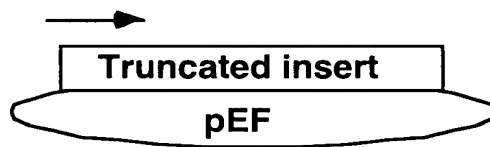
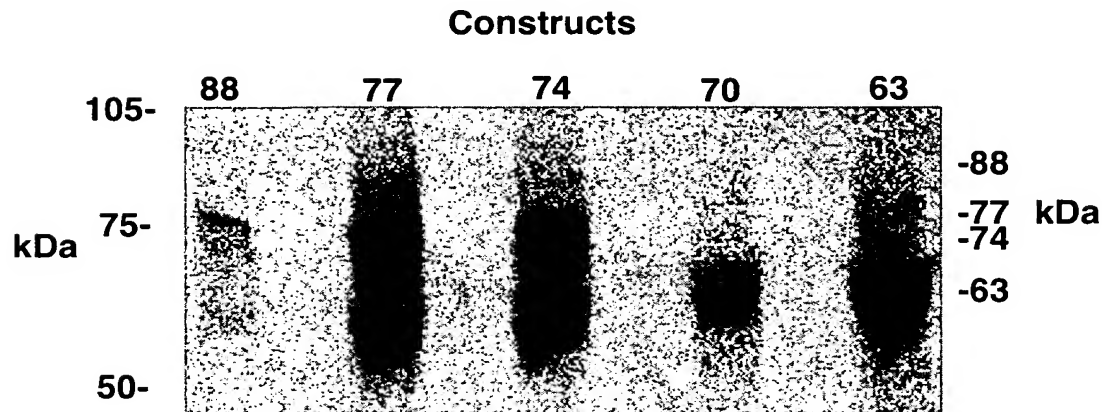
Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

FIGURE 29

40/61



Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.
Docket No.: 15060-42
Gordon F. Sieckmann, Phone 314-621-5070

41/61

FIGURE 30



iPLA₂ Gamma Functional Domains

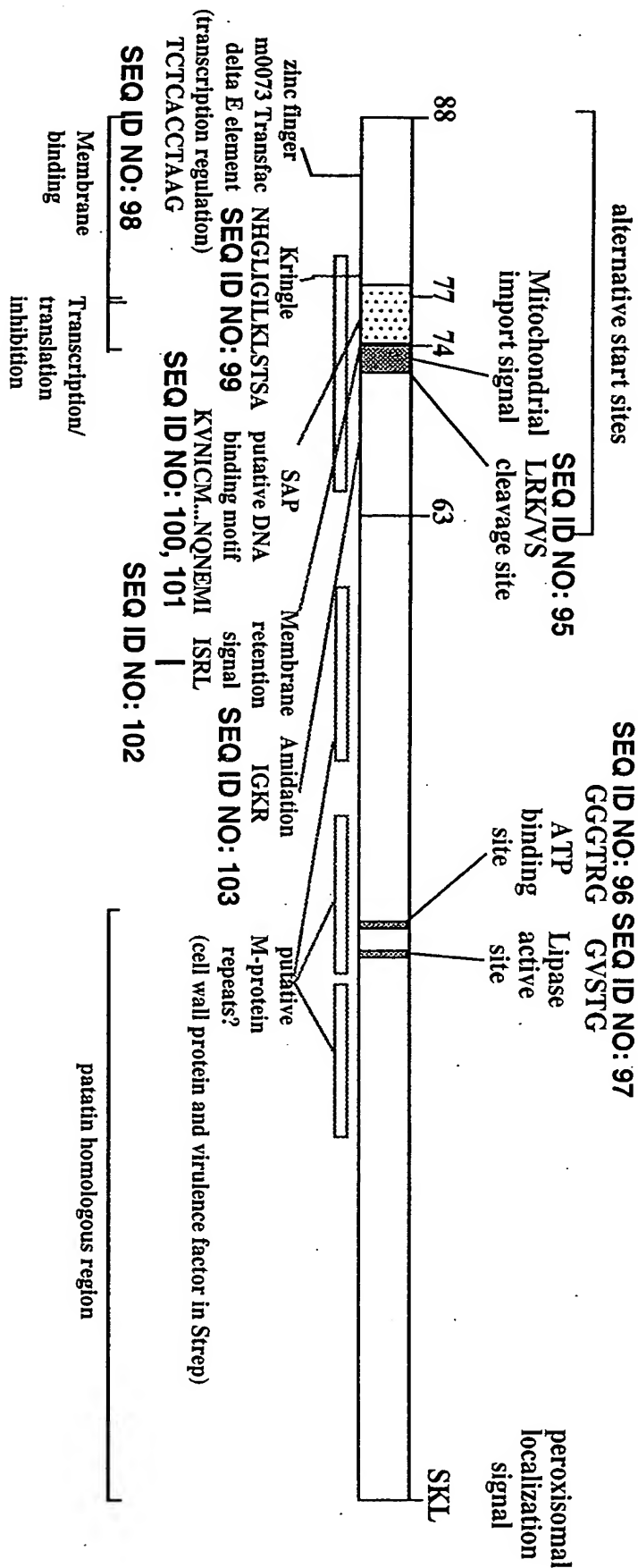


FIGURE 31

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE $A_2\gamma$
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

43/61

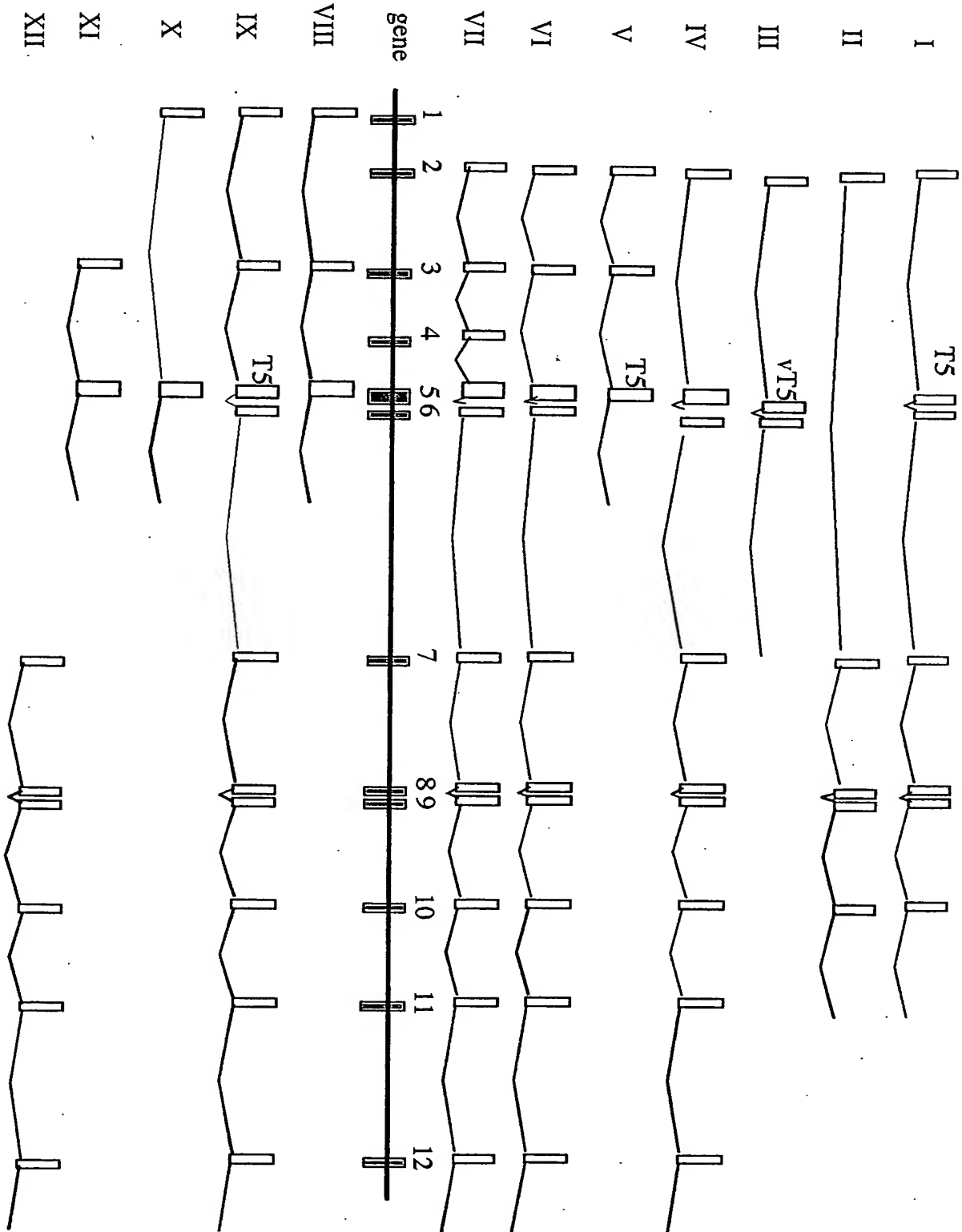
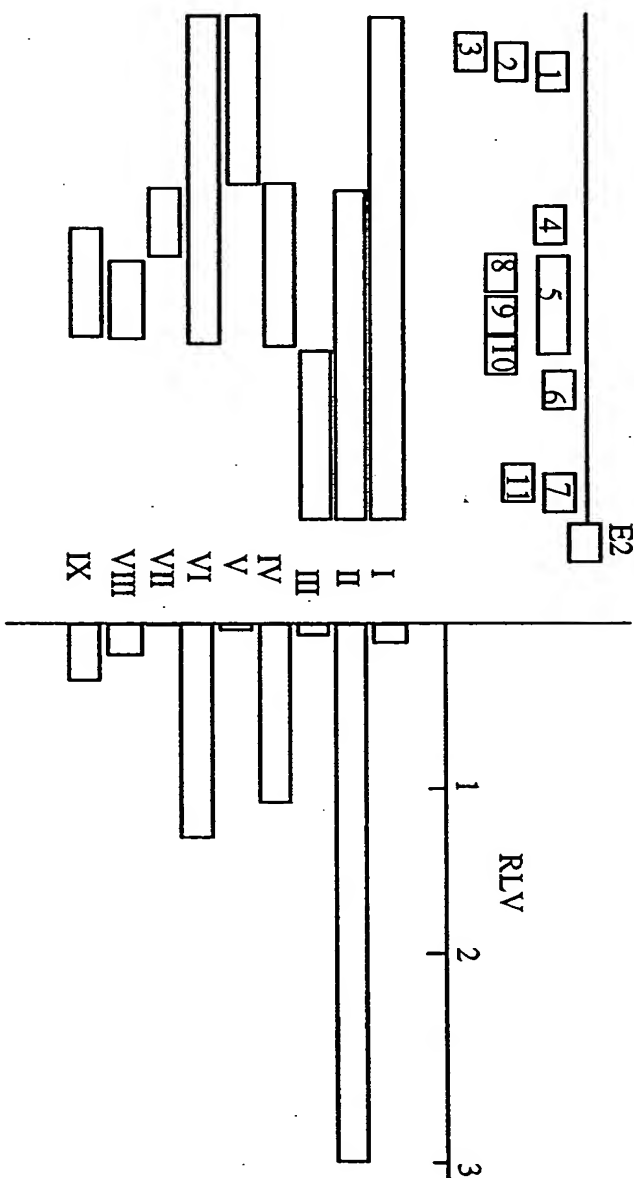


FIGURE 32

FIGURE 33

Promoter Analysis of iPLA₂ γ Pre exon 2



Conclusion: sequence upstream of exon 2 has promoter activity. Enhancer activity resides in the region 200-400nt upstream of exon 2 (fragment IV). This region contains a CACG VNTR like sequence as well as sequences that match consensus sites for Sp1 (8), GATA1 (9), p300 (4), and Gcrl (10). GC regions upstream (1) and downstream (7) of this positive promoter region commonly are negative regulatory elements. Truncated fragments (II and VI) each lacking a GC region have enhanced promoter activity while fragments (III and V) containing the GC regions but lacking region IV have minimal promoter activity. Presumably both GC regions are required for maximal inhibition. Region IV may have less than optimal promoter activity if positive promoter elements are immediately upstream or downstream of region IV.

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

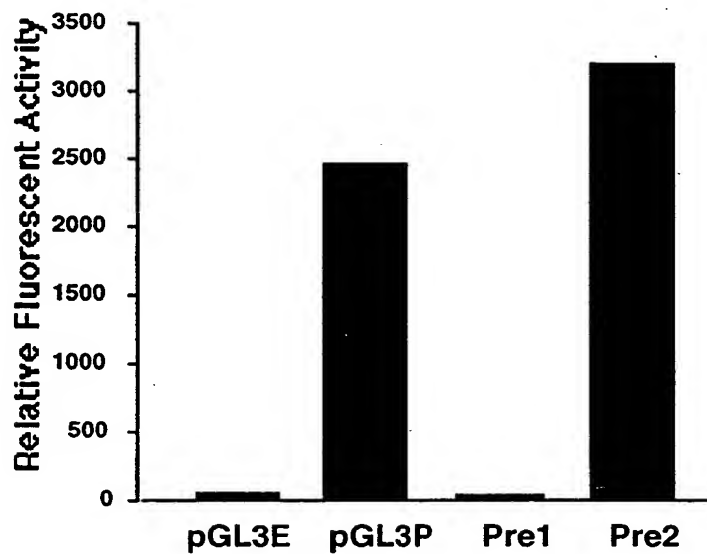
Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

45/61

FIGURE 34. Promoter Activity of Pre Exon 1 and 2 Regions



Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

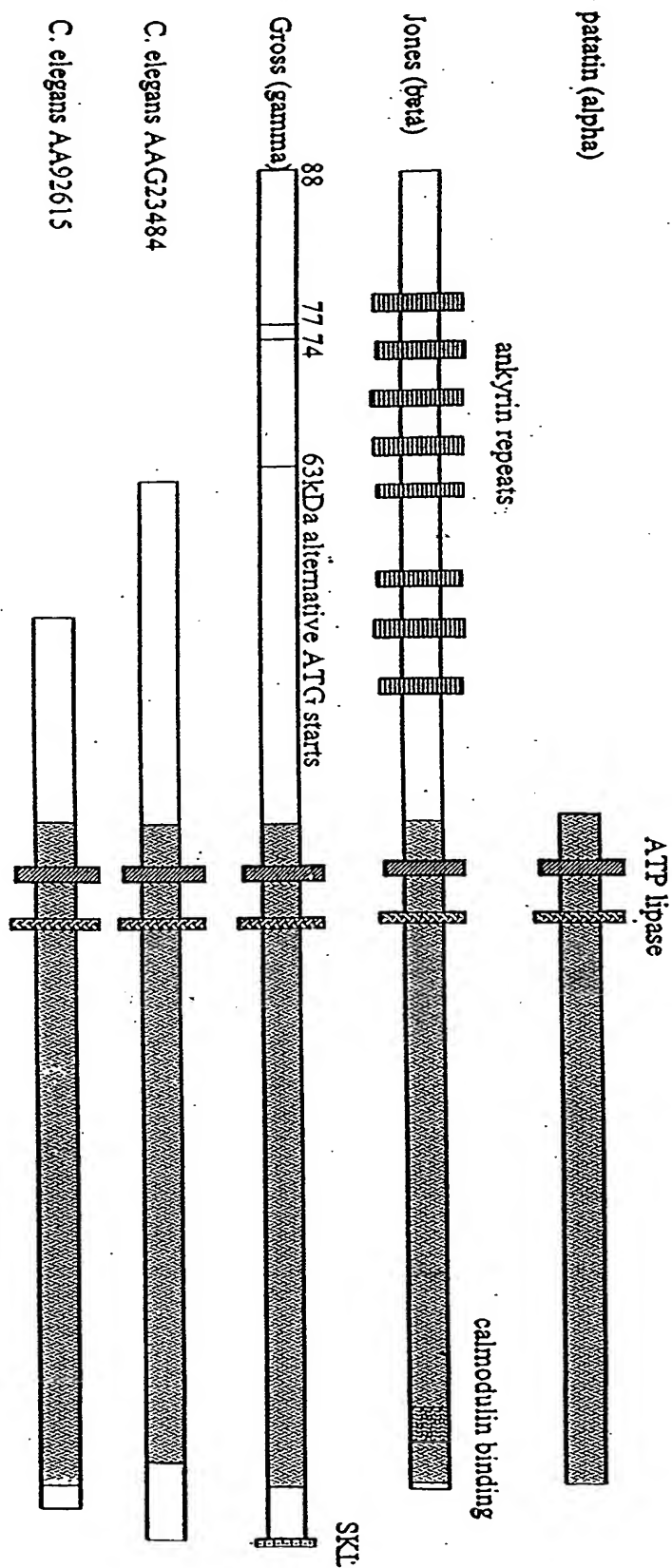
Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

46/61

FIGURE 35



Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

47/61

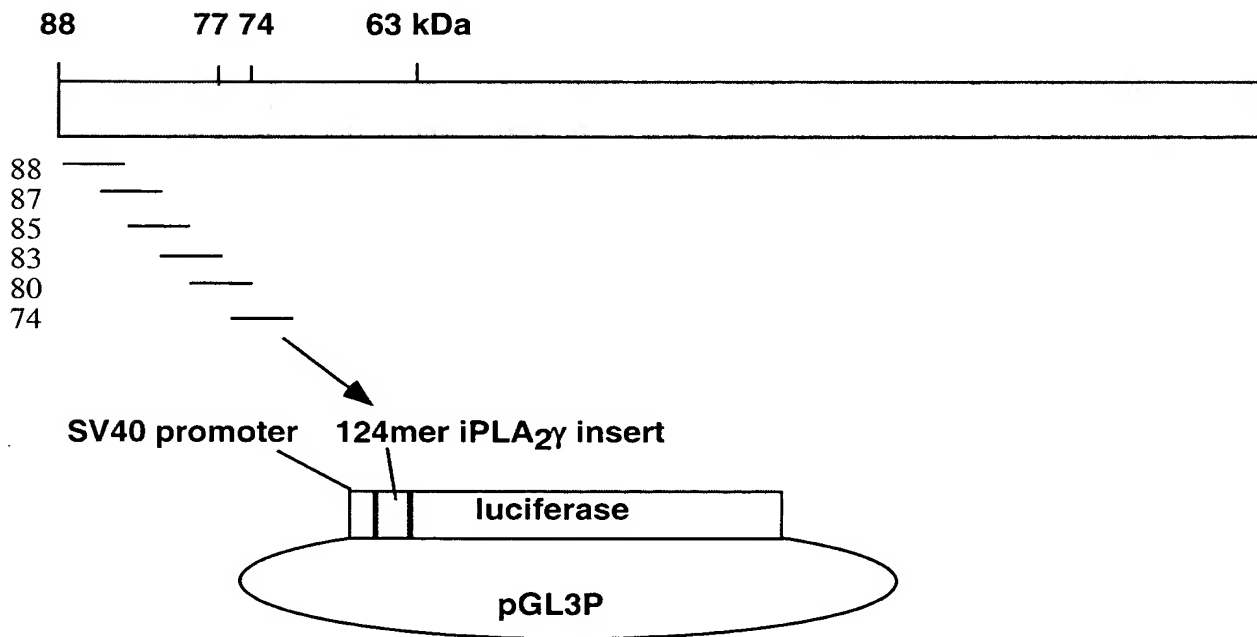
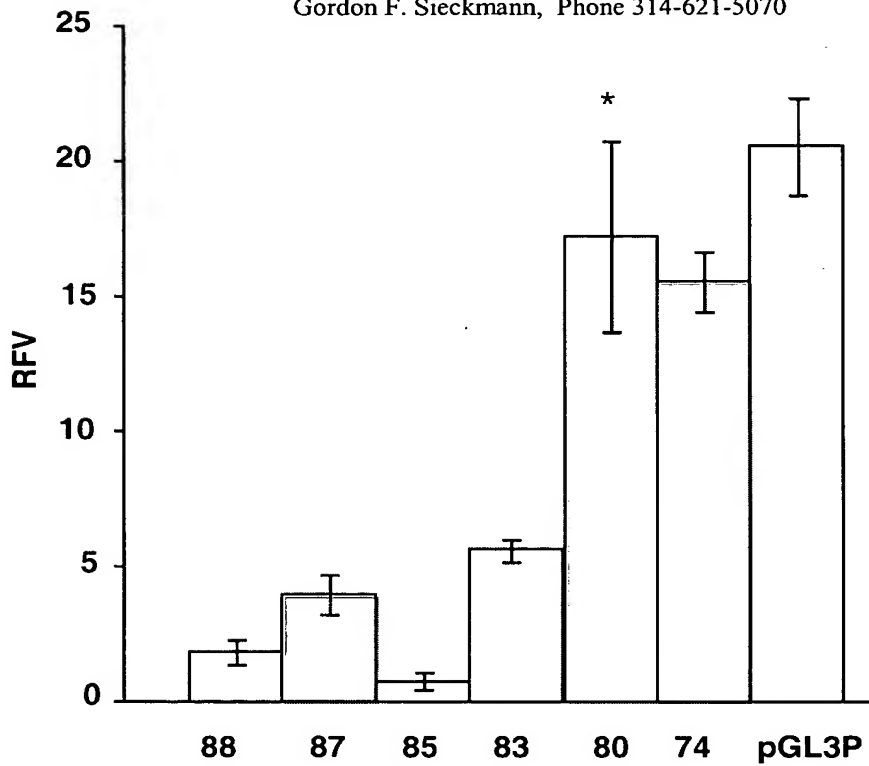


FIGURE 36

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.
Docket No.: 15060-42
Gordon F. Sieckmann, Phone 314-621-5070

48/61

FIGURE 37



Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

49/61

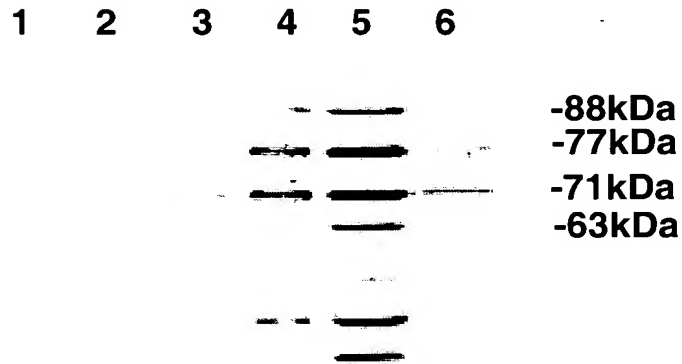


FIGURE 38

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.
Docket No.: 15060-42
Gordon F. Sieckmann, Phone 314-621-5070

50/61

FIGURE 39

Alignment of Mouse, Rat, and Human Pre-exon 2 Sequence

Mouse	BCGTCACCTCCGCTGGGGGCGCGCGCGCTAGCGG-----TGGGTGTG--CTGGTCAC--GCCAGTGTTTGGGT	88
Rat	BCGTCACCTCCGCTGGGGGCGCGCGCGCTAGCGG-----AGGTGAGG--CTG-TAGC--GCCAGTGTTTGGGT	89
Human	BCGTCACCTCCGCTGGGGGCGGAGCGGGGCGGGCTGAGTGGGTGCGACCTAGCTGCTGCGCCAGTGTTTGTGTT	90

Inr

L

L

SEQ ID NO:

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

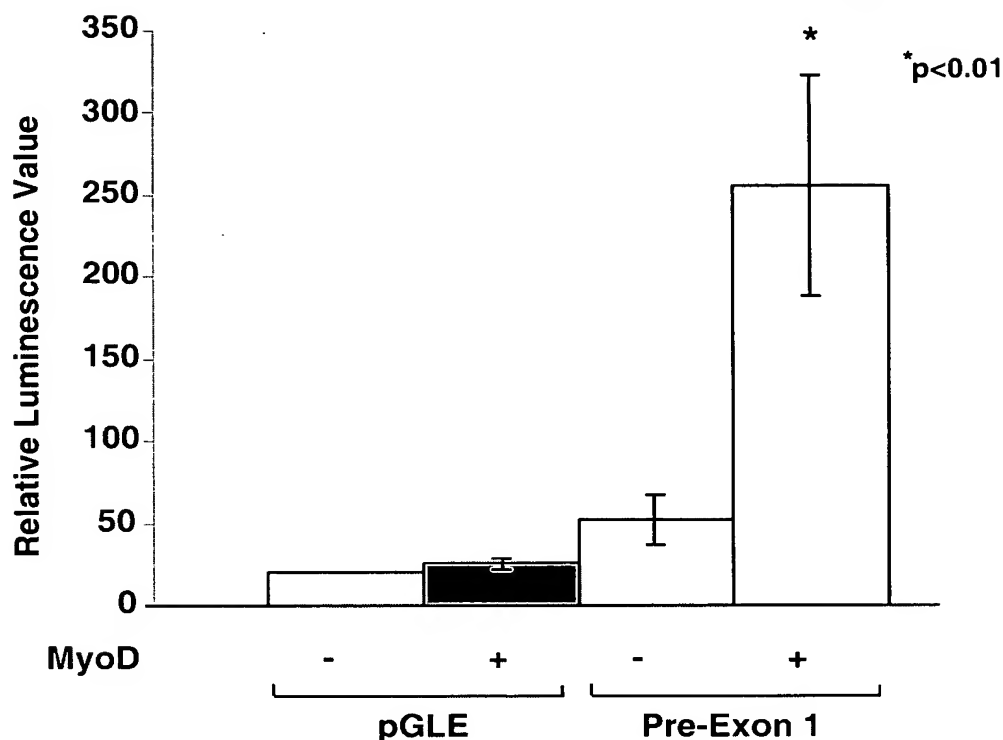
51/61

FIGURE 40



FIGURE 41

MyoD Stimulation of Promoter Activity in Pre-Exon 1 Sequence of iPLA₂γ



Putative MyoD Elements within Pre-Exon 1 of iPLA₂γ

```

-395                                     -336
ATTATAATACTGTGCAGCAACGGCAATAAGAGAAGTGAGCACAGGTGGAAGGAATGATTC
                                     GGACAGGTGGG
                                     E-box (MyoD)
-335                                     -138
ATTCTATGAGTAGTGAGGTAAGATTTTCTGGCTGAAGGACAAACAAATCTTTAGGAGGA
-137                                     -77
CAAGGTGGAAGGGGAGCTAAGCCAACAGCATGACCAAGGCACTAAGTATGAAAAGCAACA
-76                                     -17
AGAGTATCTGGGGAAGTACAGGTGTGGCTGCAGGATAGAGAGTCAGAGGCAAGTGGTGAA
      CACAGGTGGTG                      CGACAGGTGGTG
      E-box (MyoD)                    E-box (MyoD)
-16      -1      1
AGTAAAGGCTGGAAGG TCAGCAGGGTCAGA
                        Exon 1
  
```

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE $A_2\gamma$
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

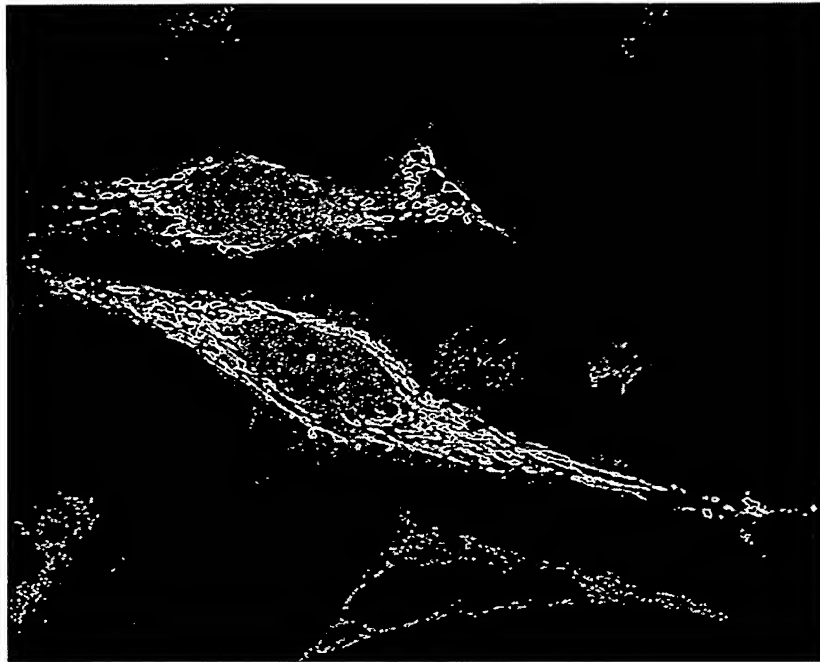
Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

53/61

FIGURE 42A



Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

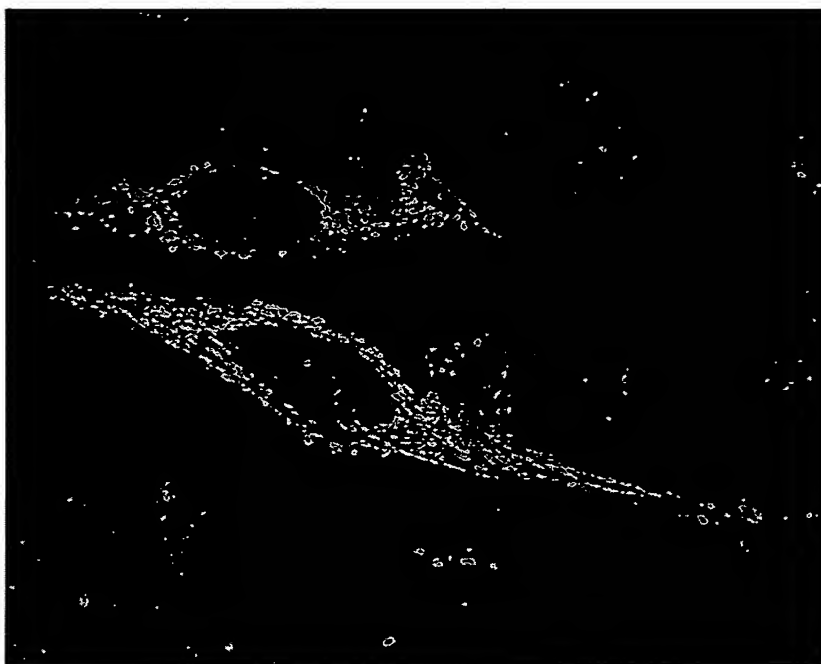
Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

54/61

FIGURE 42B



Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

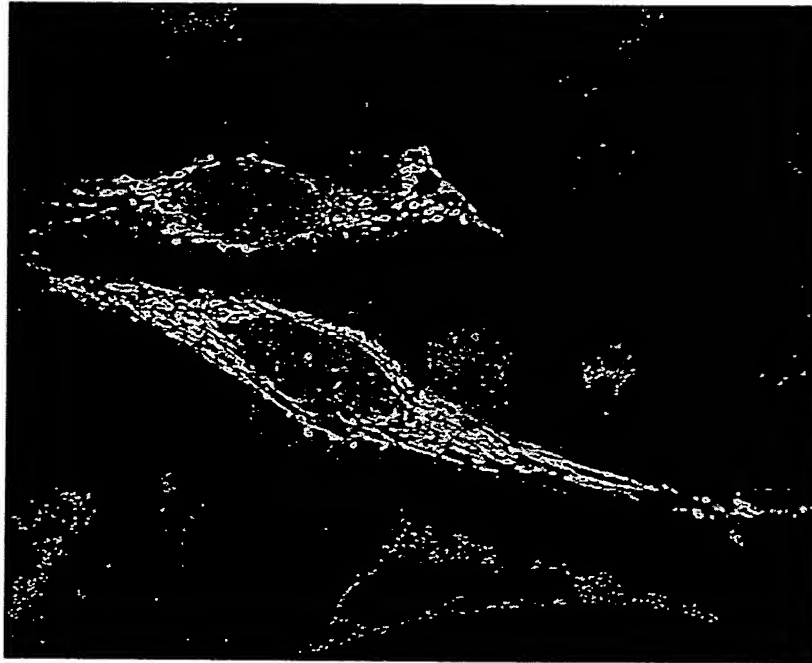
Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

55/61

FIGURE 42C



Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.
Docket No.: 15060-42
Gordon F. Sieckmann, Phone 314-621-5070

56/61

Table I

Exon Number	Length (nt)	5' Intron Sequence	Corresponding AC005058 Residues	Sequence	3' Intron Sequence
----------------	----------------	--------------------------	---------------------------------------	----------	--------------------------

1 346 GGAAGG 135622-135327
GTATGG
SEQ ID NO: 29
TCAGCAGGGTCAGAACCTATAATTTCATTCCGTATATTCT

GTGAAGATGTACAGCCAGCAAAAGCTTTTAAATTCGGGAA
AACACGATTGGACTTGCACTTCAAAAAGATTACCGTGTT
GCACAGAAGAGACTGACTGGGTCAGAGGTTAGTTACAGGC
TGAAAAACCAAGTTAGATGAAACTGAAGAGCAAGATGAA
AGCCTGAACCTAGAGCAGTGAATGCCAATGTGAGCAGA
GGAACGATTCAGAATTTCTGCCGTAAACTCATCAGACT
TCATGACTGATTAAAG

SEQ ID NO: 30

TTTGGCCTTCTAGAGTGTATATACAGCTGGAATCATACTG

4 112 TCATAG 125571-125460
GTAGGT

CTATGTCGGAATGTTGTGCTCTCAAAATTCATGTGA
AATCATTAACCGCTAAAGTGATGTATTAAAG

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

57/61

Table II
iPLA₂γ Splice Variants

Variant	Exons ESTs	Clone	Source
I	e2/TT5BI562455		testis
II	e2/T7 AL59775		unknown
III	e2/t5 BI333454	462	cervix heart
IV	e2/5 AF263947 BI596690; BI333453; BI553295 BG706376; BG708220 BG392963 AB041261	478 500	heart hippocampus hypothalamus testis T lymphocyte smooth muscle
V	e2/3/t5 BG699526; BG699526; BI550880 AL529506 R64045	507	hippocampus neuroblastoma smooth muscle
VI	e2/3/5 AA143503 BG502179 BG613307; BG701929; BG702929; BI547339 BG613307; BG719485; BG502179; BG771750 BG719485; BG613307	460.7	colon embryonal carcinoma hippocampus testis
VII	e2/3/4/5 BG723923	502	testis heart
VIII	e1/3/5 AV747051; AV747330		pituitary

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.
Docket No.: 15060-42
Gordon F. Sieckmann, Phone 314-621-5070

58/61

Table II (Continued)

Variant	Exons ESTs	Clone	Source
IX	e1/3/t5 AU136710; AK024335	492 467 485; 490	placenta HUVEC pancreas smooth muscle
X	e1/t5	494 466 460.1	smooth muscle myocardial pancreas
XI	e3/5	pan ap2/466.5	pancreatic

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.

Docket No.: 15060-42

Gordon F. Sieckmann, Phone 314-621-5070

59/61

Table III

<u>Construct Primer pairs 5' to 3' Sequence</u>		<u>SEQ ID NO:</u>
88	88f GTTGAAGCTTGTGTCTAATTAATCTGACTGTA	63
	88r TAGACCATGGTGGCTTATCCTCCAGTAATGC	64
87	87f GTGTAAAGCTTGAAGCAGAGAAGCAAGCAACTG	65
	87r ACTGCCATGGTGGCCCTTCACTTTGGTCCATTAC	66
85	85f TGGAAAGCTTGGCCACATCAGTCTACAAG	67
	85r TGCTCCATGGTGGCATCCCAATATGTAAACCA	68
83	83f GAACCAAGCTTGAAGCAGACATCTTGCAGTAAGCA	69
	83r CAAAACATGTTGGCTACGGGACATACAAATGTTCA	70
80	80f GTTGAAGCTTTTGAAACTTAGCAGTCTGTC	71
	80r ATTCCATGGTGGCTGAATAATCATTTTCATTTTGATTGCC	72
74	74f TCAAAAAGCTTATGATTTACAGTTTAGCTC	73
	74r CTTTCCATGGTGGCTGTCACATAATTTTTC	74

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.
Docket No.: 15060-42
Gordon F. Sieckmann, Phone 314-621-5070

60/61

Table IV

Splice Variant	5' Exon Number	3' Exon Residues	5' Intron Sequence	3' Intron Sequence	5' Truncated Exon 5 Sequence	SEQ ID NO:
X	1	GATTAAAG	gtatggtggt	acctccttag	TAATGCAAG	75, 76
III	2	GCATCCCG	gtaagtaggc	acctccttag	TAATGCAAG	91, 92

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

Inventor: Richard W. Gross et al.
Docket No.: 15060-42
Gordon F. Sieckmann, Phone 314-621-5070

61/61

Table V
Sequence surrounding iPLA₂γ ATG Start Sites

<u>Isoform</u> <u>NO.</u>	<u>Sequence</u>	<u>Matches to Kozak Consensus</u>	<u>SEO ID</u>
88kDa	T T T T A A G T T A T G T	1/12 adequate	79
77kDa	A A C A T T T G T A T G T	1/12 poor	80
74kDa	C A A A A T G A A A T G A	1/12 poor	81
63kDa	A A G G A A A A A T G T	2/12 adequate	82
consensus	G C C G C C A C C A T G G G		